دورة التحاليل الطبية

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Introduction to laboratory medicine

Clinical Laboratory Science

Clinical Laboratory Science, also called Medical Technology, is the health profession that provides laboratory information and services needed for the diagnosis and treatment of disease. ***Clinical Laboratory Scientists*** perform a variety of laboratory tests, ensure the quality of the test results, explain the significance of laboratory tests, evaluate new methods and study the effectiveness of laboratory tests.

Medical laboratory departments

Laboratory medicine is generally divided into two sections, and each of which is further divided into a number of units. These two sections are:

Histopathology laboratory
Clinical pathology laboratory

Clinical pathology laboratory is divided into the following unites:

➤ Clinical Chemistry unit	➤ Clinical Microbiology unit	➤ Clinical Hematology unit
➤ Clinical immunology unit	➤ Cytogenetics unit	➤ Molecular biology unit

Why laboratory tests are ordered?

- To screen for disease (screening tests as ESR , CBC)
- To confirm a diagnosis
- To exclude a disease that is possible but not likely
- To aid differential diagnosis, e.g. to distinguish between different forms of jaundice.
- To assess the severity of disease, e.g. plasma [urea] or [creatinine] in renal disease.
- To determine risk factors for disease as lipid profile
- To monitor progress of disease, e.g. plasma glucose to follow of patients with diabetes.
- To monitor effectiveness of disease treatment as in case of anticoagulant therapy.
- To detect complications or side-effects of drugs e.g. aminotransferase measurements in patients being treated with hepatotoxic drugs.

Laboratory procedures

Laboratory procedures divides into three stages:

Pre-analytical stage: which covers the following

- Reading the request form
- Recording the patient data and medical history
- preparation of the patient
- collection and transport of the specimen

البيانات الشخصية الواجب توافرها لمريض المعمل

- **الاسم ثلاثي** : حتى لا يحدث خطأ أثناء أخذ العينات من حيث الأسماء.
- **رقم التليفون** : حتى يتثنى للمعمل الاتصال بالمريض للاستفسار عن نوع علاج أو زيادة جرعة خصوصً في تحليل سيولة الدم.
- **الســن** : لأنه يوجد لكل سن نسب طبيعية في الدم.
- **الطبيب المعالج** : حتى يتثنى للمعمل الاتصال بالطبيب المعالج للاستفسار عن طبيعة تحليل أو نوع علاج أو تشخيص الحالة.

اسئله هامه لمريض المعمل

- **عدد ساعات الصيـام** : لأنه يوجد تحاليل تحتاج إلى صيام 12 ساعة مثل دهون الدم مثلاً و 8 ساعات مثل السكر.
- **هل يؤخذ علاج للسكر** : إذا كان المريض يريد عمل اختبار سكر.
- **هل يؤخذ علاج لسيولة الدم** : إذا كان المريض يريد عمل اختبار لسيولة الدم خاصة , PT , PTT
- **هل يؤخذ علاج للغدة** : إذا كان المريض يريد عمل اختبارات الغدة.
- **هل يؤخذ مضاد حيوى** : إذا كان المريض يريد عمل مزرعة.
- **هل سبق نقل دم** : وذلك لعلاقة الدم بنسبة الهيموجلوبين أو فيروسات الالتهاب الكبدى أو الإيدز ومتابعة نسبة الهيموجلوبين و. G6PD
- **أول يوم في أخر دورة** : وهذا مهم في حالة اختبارات الهرمونات أو الحمل.
- **هل تم إجراء أشعة بالصبغة أو بالمواد المشعة :** وذلك لأن الصبغة تؤثر علي تحاليل البول والكيمياء العادية أما المواد المشعة فتؤثر علي الهرمونات.

Analytical stage : which covers the following
- checks the test method, the reagents, standards, and control materials
- checks the equipment and instruments used
- perform the test and calculate the results

Post-analytical stage: which covers the following
- Reporting the test result which must include
  1. Test name and type of specimen analyzed
  2. Test results clearly
  3. The measurement unit and reference range (Review the range with the age and sex)
  4. The comment if found
- Interpretation of test results to follow if the result is seriously abnormal or unexpected.

Laboratory Safety Rules

- Laboratory should be designed in such a way that you can work safely:
  1. it must be easily cleaned.
  2. contains a sink for washing.
  3. Illumination is adequate for all laboratory activities.
- Treat all body fluids as a potentially infectious materials.
- A lab coat and gloves should be worn during laboratory work.

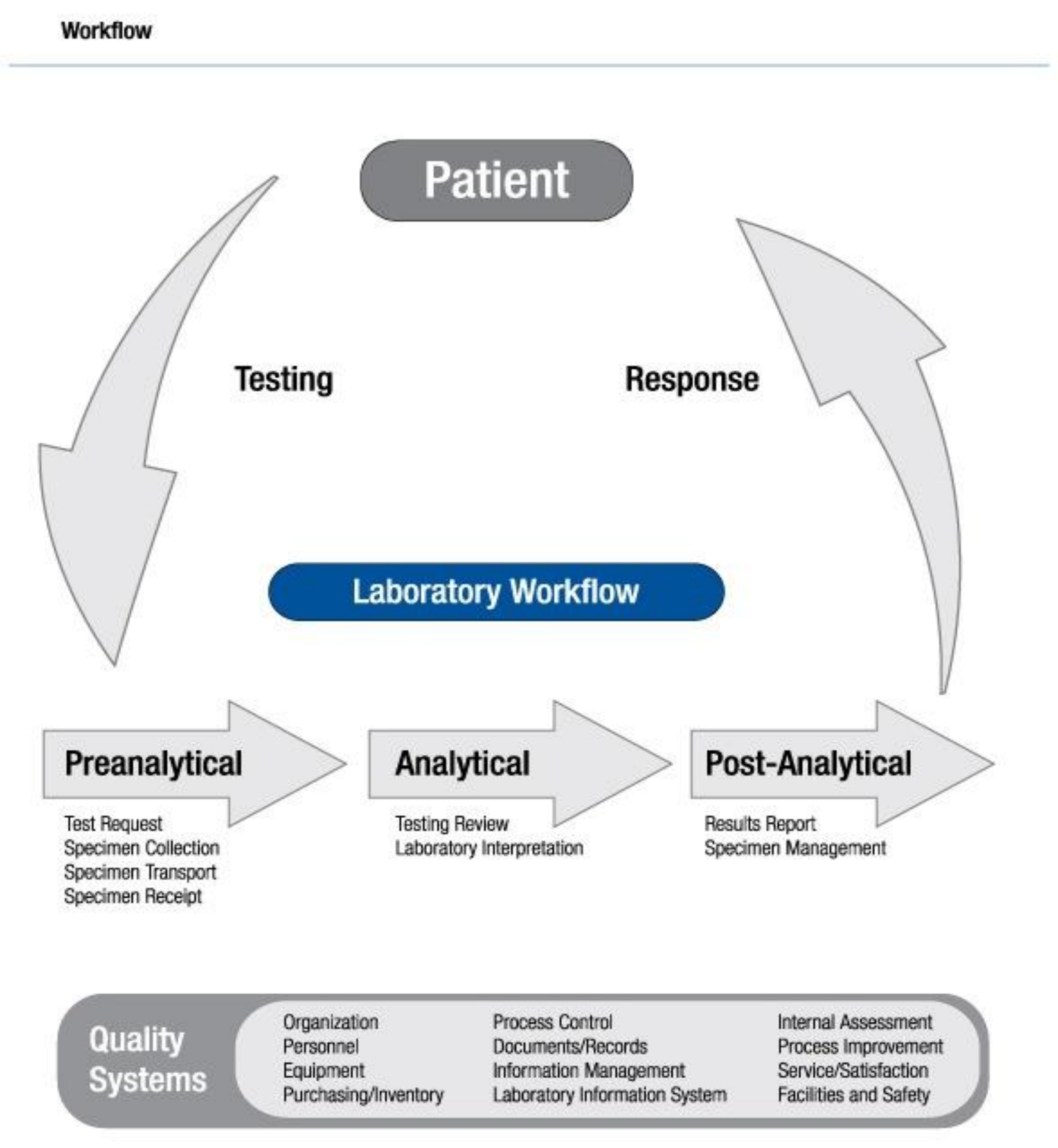
Note to remove gloves before using telephone

- Do not eat food, drink, or chew gum in the laboratory.
- Do not use laboratory glassware as containers for food or drinks.
- Work areas should be kept clean and tidy at all times.
- Do not place contaminated pipettes on the bench top.
- Keep hands away from face, eyes, mouth, and body while using chemicals or lab equipment.
- Wash your hands with soap and water after performing all experiments.
- Never use mouth suction for chemicals or body fluids
- Keep fingers, pencils, loops etc. out of your mouth.
- Perform adequate sterilization before washing or disposing waste.

Needle stick injuries

The 'first aid' instructions for personnel in case of needle stick injury should contain at least following:

- Don't panic. Make sure that injury does not happen again.
- Clean the infected area:
- Rinse with substantial amount of water and soap.
- Don't squeeze wounded area (the practice to "milk out" more blood is controversial and not recommended).
- If you have blood on eczema or on puncture wound, place a patch with alcohol (at least 70% alcohol) over it for two minutes.



Blood sampling

Anticoagulants

It is the substances that prevent coagulation of blood and it is used to prepare a plasma sample in laboratory.

What is the difference between plasma and serum samples?

Serum refers to the clear liquid that separates from blood when it clots ,serum do not contain prothrombin or fibrinogen but it contain thrombin that is formed during blood clotting so it cannot be used in blood transfusion. Plasma is the pale yellow liquid obtained from centrifugation of blood with anticoagulant; it contains clotting factors and has no thrombin.

EDTA (Ethylene diamine tetraacetic acid)

Preparation

Found as disodium salt or dipotassium salt (K salt is more soluble so it is more preferable)

Dissolve 1gm of powder in 100 ml DW then use 50 µl for each 1ml blood

Mechanism of action

It acts by binding calcium ion that is essential for blood clotting to form insoluble salt.

Uses

Must be used in tests that need whole blood such as CBC, blood group, Rh, ……

Advantage

- Cellular elements are preserved well
- Leukocyte stain ability is excellent
- EDTA prevent platelets clumping

Disadvantage

- EDTA is not suitable for coagulation tests as PT
- EDTA inhibit the action of some enzymes as amylase enzyme
- Excess EDTA will cause shrinkage of the blood cells

Sodium citrate

Preparation

Dissolve 38 gm in 100 ml DW (it is used as 3.8% solution)

Mechanism of action

It converts ionized Ca into unionized soluble complex but not as strongly as EDTA

Uses

The most popular tests use citrate is:

ESR test (400 ml citrate + 1.6 ml blood)

PT & PTT test (200 ml citrate + 1.8 ml blood)

Advantage

- Its action is reversible so it can be used in coagulation studies

Disadvantage

- When used in a dry form it cause cell shrinkage
- It cause blood dilution when used as solution so it is not suitable for some tests as Hb

Sodium fluride

Mechanism of action

Sodium Fluoride has a weak anticoagulant action through the formation of a weakly dissociated Ca complex

Uses

It is used as a powder 5 mg/ml blood for glucose determination

Advantage

Sodium fluoride prevents glycolysis for 3 days by forming an ionic complex with Mg++, thereby inhibiting the Mg++ dependent enzyme, enolase of the glycolytic pathway, preventing the consumption of glucose by the RBC's if blood is left standing at room temperature.

Disadvantage

- Very poisonous
- It is poorly soluble so blood must be mixed well

Heparin

Mechanism of use

It facilitates the action of anti-thrombin III which is a protease that inhibit active factors (IX, X, XI, XII) so inhibit conversion of fibrinogen to fibrin and Inhibits thrombin formation.

Uses

It is used in concentration of 10-50 U/ml (0.1 ml /5ml blood)

Advantage

- Some samples must be collected on heparin such as Blood gases, and D-dimer
- Heparinized plasma is preferred for potassium measurements to avoid an elevation due to the release of potassium from platelets as the blood clots.

Disadvantage

- More expensive
- Blood should be examined with in 8 h (because it prevent coagulation for only limited period)
- Can not be used for coagulation testing.
- It cause platelets aggregation so it is not recommended for CBC

Vein puncture (venesection, phlebotomy)

Preparation for blood collection

- Read the request of the patient.
- Verify the patient's condition. Fasting, dietary restrictions, timing of the test, and medical treatment (especially in blood glucose test and PT test).
- prepare equipments for blood sampling (Tourniquet, Alcohol, Cotton, Adhesive strip, Sterile Disposable syringes)
- Estimate the blood volume to take.
- Prepare the suitable tubes for each test.

Pre-Collection Variables

In preparing a patient for phlebotomy, care should be taken to minimize factors that might influence laboratory determinations. These include:

Diurnal variation

The cyclical variations in body chemicals due to light/dark, or sleep/wakefulness include:

- Iron peaks early to late morning; decreases up to 30% during the day
- Cortisol peaks 4–6 a.m.; lowest 8 p.m. to 12 a.m.; 50% lower at 8 p.m. than at 8 a.m.
- Prolactin higher levels at 8 and 10 p.m.
- LH and FSH in women during menstrual cycle

Time of Collection

Sometimes, samples have to be collected at a specific time. Failure to do this can lead to erroneous results and misinterpretation of a patient's condition. For example:

- Physician may want to monitor a cardiac marker to determine if it is rising or decreasing.
- In therapeutic drug monitoring, trough and peak levels of a drug may be ordered. Trough specimens reflect the lowest level in the blood and are generally drawn 30 minutes before the drug is administered. The peak specimen is drawn shortly after the medication is given.

Exercise.

- Pnhysical activity has transient and long-term effects on laboratory determinations. Exercise may elevate ***creatine phosphokinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH)***,.
- During strenuous exercise the increased production of lactic acid will compete with uric acid for renal excretion so serum uric acid concentration increases.

Stress.

- Total cholesterol has been reported to increase with stress, and high-density-lipoprotein cholesterol (HDL) to decrease as much as 15%.
- Hyperventilation affects acid-base balance, and elevates leukocyte counts.
- Stress cause high glucose and enzyme activity due to adrenal stimulation

Diet.

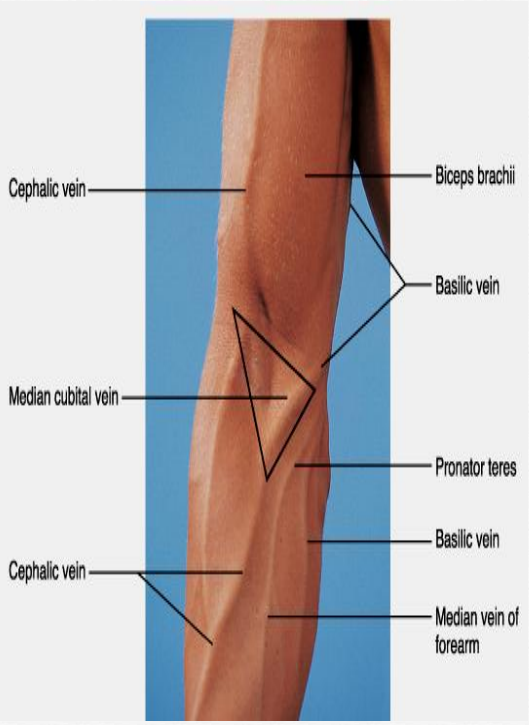
An individual's diet can greatly affect laboratory test results. The effect is transient and easily controlled.

- Glucose and triglycerides, absorbed from food, increase after eating.
- High protein, low carbohydrate diets, greatly increases the serum BUN and uric acid.

Positioning the patient and choosing the vein

- The patient should sit Comfortable in a chair or sit up in bed.
- In order to avoid problems with hemoconcentration and hemodilution, the patient should be seated for 15 to 20 minutes before the blood is drawn.
- Patients should not stand or sit on high stools because of possibility of fainting.
- The patient arm extend from shoulder to wrist and arm should not bent at elbow.
- Avoid arm with burn area, hematoma , scaring , recently injected or withdrawn syringe and avoid edematous extremities (tissue fluid accumulation alters test results).
- Avoid arm on the side of a previous mastectomy due to patient with a mastectomy may have lymphostasis on the effected side, making the patient highly susceptible to infections, also lymphostasis affect blood composition and test results.
- The vein selected should be large, straight that does not roll, readily accessible, and sufficiently close to the surface to be seen and palpated.
- In adults the larger and fuller median cubital, basilic and cephalic veins of the arm are used most frequently.
- dorsal hand veins can be used but it is not suitable in patients with poor circulation

If the vein is difficult to be seen The following methods can be done to help:



- Ask the patient to make fist this helps make the vein more prominent.

  Note *Repeated fist clenching with or without tourniquet causes excessive release of potassium from skeletal muscles (pseudohyperkalemia)*
- You can force blood into the vein by massaging the arm from wrist to elbow
- Apply a warm, damp washcloth to the site for 5 minutes this increases the blood circulation, which makes it easier for the phlebotomist to find a vein.
- Drinking water helps blood flow better and makes the veins more likely to stick up and be found easily (even if the patient is fasting)
- Apply tourniquet to distend the vein (tourniquet obstructs the venous return so it helps to distend the vein) .

As a rule, the tourniquet should not be placed too tightly (as to stop arterial flow) or left on the patient for more than 2 min.

<u>What are the effects if it left for more than 2 min?</u>

Prolonged application of the tourniquet results in partial stasis of blood which leads to *hemoconcentration* that increase concentration of serum enzymes, *potassium, proteins*, and protein-bound substances as calcium.

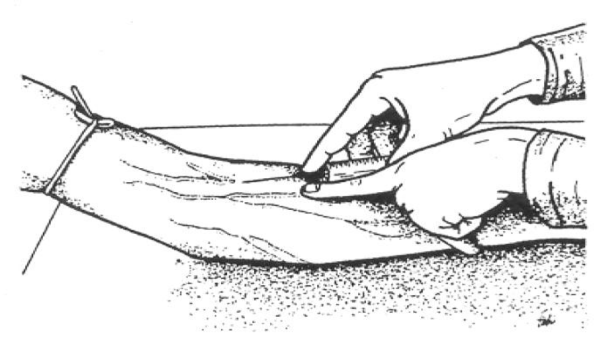
VeinViewer

VeinViewer is a direct projection vascular imaging device clinically proven to improve peripheral vascular access. VeinViewer uses harmless near-infrared light to produce a digital image of a patient's veins and project it directly on the surface of the skin. VeinViewer projects an image much like a picture on a movie screen, but VeinViewer does this in real time making the patient's skin the "screen".

This innovative vascular access tool enables clinicians to see vessels up to 10mm deep and potentially find more options for access than by traditional methods along.

Venipuncture with syringe

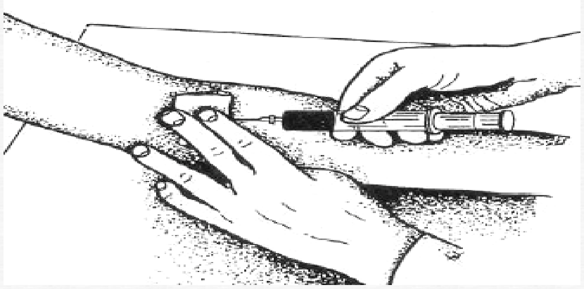
1. Check the syringe
   - Check to be sure that the syringe works smoothly by pressing the piston.
   - The plunger must be pushed firmly to the bottom *of the cylinder to prevent injection of air into the vein, this can be fatal.*



2. Use 70% alcohol as disinfectant the site in concentric circle and let it to dry for 30–60 sec to avoid hemolysis and burning sensation.
3. Palpate the vein by left hand. It should rebound
4. Fix the vein by drawing skin tight over the vein to prevent vein from moving
5. Enter by the needle at 45 degree angle (under the skin and then into the vein)

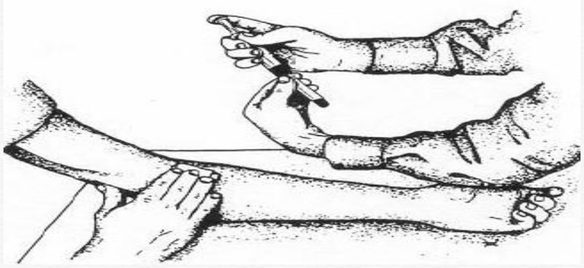
When the needle enter the vein there is sudden lose of resistance and blood come in the head of needle

6. Remove the tourniquet once the needle has been inserted



   Note *If the needle were removed prior to the Tourniquet being removed, blood would be forced out of the venipuncture site, resulting in hematoma.*

7. Withdraw blood gradually by gently pulling upon the syringe plunger



   Note *Vigorous pulling on the plunger of the syringe can collapse the vein, produce hemolysis of the blood specimen.*

   Note *Take too much time in obtaining blood cause sample clotting*

8. Place a sterile cotton piece over the point where the needle entered the skin.
9. Remove the syringe quickly
10. Dispose of contaminated materials and needles in special disposal containers.

Control bleeding

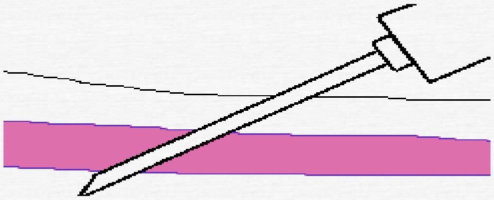
- Patient press cotton for 5 min to stop bleeding
- do not bend the arm to avoid hematoma
- Raise the arm above the heart level for approximately 3–5 min to control bleeding, if necessary.

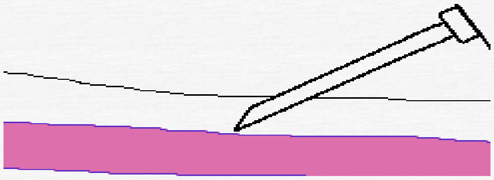
Fainting subjects

If the subject looses consciousness or feels dizzy during the procedure, it should be discontinued. The subject should be asked to place his/her head between their knees. He/she should subsequently be asked to lie down. If the subject is willing for the test to be continued after a suitable length of time, the blood sample could be taken. Otherwise the possibility to give the blood sample at a later time could be discussed.

What I do if the blood sample is unobtainable?

1. Change the position of the needle.
   - If the needle has penetrated too far into the vein, pull it back a bit.
   - If it has not penetrated far enough, advance it farther into the vein.
2. Loosen the tourniquet. It may have been applied too tightly, thereby stopping the blood flow.
3. Re-anchor the vein. Veins sometimes roll away from the point of the needle and puncture site.





What I do if the blood stops flowing into the tube?

The vein may have collapsed, this is usually occurs in smaller veins and veins of geriatric patients due to:

- withdrawing too quickly with syringe
- inappropriate needle selection

In this case resecure the tourniquet to increase venous filling. If this is not successful, remove the needle, take care of the puncture site, and redraw.

Blood collection from cannula

In general, blood should not be drawn from an arm with a cannula because intravenous therapy (IV) fluid may dilute the specimen or give false results.

Otherwise follow these procedures:

1) Stop any infusion into the cannula for at least 10 minutes.
2) With a syringe attached to the hub of the cannula, gently aspirate 1 ml then discard it as it is contaminated with infusion fluid.
3) With a syringe, aspirate the volume of blood required for your specimen.

Note *You cannot use this blood for a coagulation studies if heparin has been run through the cannula as even a trace amount of heparin in the cannula will substantially change the result.*

❖ Vacutainer System

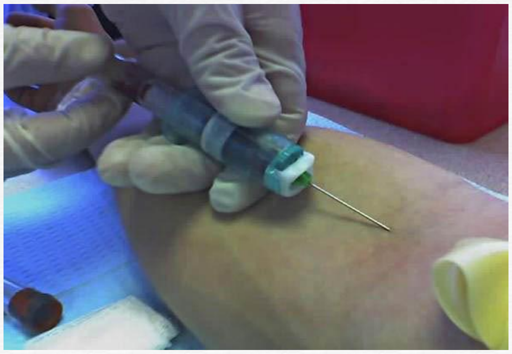
The Vacutainer System consists of:

1. A double-pointed needle (Vacutainer needle), the long end of the needle is used for penetrating the vein; the shorter end is used to penetrate the vacuum tube.
2. A plastic holder into which the phlebotomist screws the double pointed Vacutainer needle.
3. A series of vacuum tubes with rubber stoppers of various colors. The air pressure inside the tube is negative, less than the normal environment so the difference in pressure between the inside of the tube and the vein causes blood to fill the tube.

Stopper color	Additive	Uses
Red	None	Collecting serum sample
Purple	EDTA	Collecting plasma sample
Blue	Sodium citrate	PT & PTT tube
Black	Sodium citrate	ESR tube
Gray	Sodium fluoride	Blood sugar tube
Green	Lithium heparin	Collecting heparinated blood
Gold	None	Serum separator tube (SST) contains a gel at the bottom to separate blood from serum

Note *Therapeutic drug monitoring specimens should not be collected in tubes that contain gel separators as some gels absorb certain drugs causing a falsely lowered result.*

Venipuncture with evacuated tubes



1. Insert the stopper of the first tube to be drawn into the adaptor.
   Before using the tube it should be tapped gently to dissolve any additives from stopper and this prevents aspiration of additives into vein
2. Insert the needle into the vein.
3. After entry into the vein push the tube all the way into the holder; vacuum is broken, and blood flows freely into the tube.
4. Remove the tourniquet once the blood flow has begun.
5. Fill the tube until the vacuum is exhausted.
6. Remove tube from adaptor and insert subsequent tubes.
   Note *ensure the needle is not moved while tubes are being changed.*
7. Place a sterile cotton piece over the point where the needle entered the skin.
8. Remove the needle quickly
9. Patient press cotton for 5 min to stop bleeding and do not bend arm

Using syringes is more preferable when the patient's veins are small or fragile because suction from an evacuated tube can cause the vein to collapse. Using a syringe allows control of the amount of suction.

Specimen Processing

Transfer samples to test tube

1) Be sure that the tube and its stopper is dry
2) Remove the needle from the syringe
3) Pour the blood gently from the syringe at the wall of the tube

Note *the order of the tubes as following:*

Coagulation tube, ESR tube, EDTA tube, and Plain tubes

4) Mix well blood with anticoagulant if found, gently invert the tube 8–10 times to mix the additive with the blood.
5) Label the tube and write the name, tests and date

Do not forget to label the specimens at the sampling room before sending it to the laboratory

Separation of serum or plasma

Ideally, all measurements should be performed within 1 hour after collection. Whenever this is not practical, the specimen should be processed to a point at which it can be properly stored in order to preclude alterations of constituents to be measured. In clinical chemistry, plasma or serum is preferred for most determinations.

In some tests serum or plasma must be separated quickly such as glucose test to prevent glycolysis.

For plasma:

- For plasma samples, blood should be cooled and centrifuged as soon as possible and separated immediately after centrifuging then plasma transferred to a clean tube.

For serum:

- The blood samples are allowed to clot at 37 °C. for about 30 minutes
- blood samples then centrifuged and serum should be separated from clot and transferred to a clean tube.

Note *Centrifuging for 5 minutes at about 1000 g is adequate to obtain serum or plasma*

Specimen Rejection

Specimen rejection is not only costly and time-consuming, but may cause harm to the patient. It is therefore essential to thoroughly train staff in all aspects of specimen collection, transportation and processing.

various reasons for specimen rejection include:

- Hemolysis / lipemia
- Clots present in an anticoagulated specimen
- Non fasting specimen when test requires fasting
- Improper blood collection tube
- wrong sample volume
- Improper transport conditions (as in blood gases)

- Unlabeled or mislabeled specimen
- A specimen is unsuitable for testing because it has been stored incorrectly, or is too long in transit before it reaches the laboratory.
- Blood sample is taken from an arm in which an intravenous (IV) infusion is running.

Preservation of sample

Preservation is very important for good results and we must know some important rules as:

What is the suitable preservation temperature?

- Enzymes for example must be refrigerated at 4C (high room temperature will affect enzyme activity)
- Troponine and D – dimmer must be preserved at room temperature and don't freeze it
- Not storing whole blood samples in freezing compartment of a refrigerator it must be refrigerated at 4°C to prevent cellular changes affecting test results.
- For analysis of unstable constituents such as ammonia, plasma renin activity, and acid phosphatase, specimens must be kept at 4°C immediately after collection
- Some samples (as coagulation factors) must be preserved at -70°C degree

How long the sample can be preserved?

- Some samples must be analyzed quickly and cannot be preserved such as reticulocytic count , ESR, and PT test.
- Generally serum or plasma samples are stable at low temperature (refrigerator) for about one week.

What is the special preservation conditions?

- Bilirubin should be protected from direct exposure to light, which causes its breakdown

Transport of sample

- All laboratory specimens must be transported in a safe and convenient manner to prevent biohazard exposure or contamination of the specimen.
- All data related to the patient must be supplied with the sample
- To mail a specimen in the frozen state, solid carbon dioxide (dry ice) may be packed in a polystyrene container with the specimen, which can be kept frozen at temperatures as low as -70°C.

Note *it is more preferable to keep another sample in your laboratory for the same patient to use it if necessary*

Complications of blood sampling

Blood hemolysis

Hemolysis is the destruction of the encasing membrane of the red blood cell and the ubsequent release of hemoglobin into the plasma.

Causes of blood hemolysis

- **Incorrect venipuncture technique**

1. Collecting blood from a narrow vein
2. When a vein collapses can also cause some hemolysis.
3. Alcohol don't dry (it cause lysis to RBCs membrane)
4. Using excess EDTA or moisten EDTA as anticoagulant
5. Slow drawing blood from the vein

- **Mishandling of the specimen**
  6. Forcing blood through the needle into tubes
  7. Using wet tube or stopper
  8. Shaking the tube very hard
  9. Holding blood in too warm area

Types of hemolysis

Slightly Hemolysis

The serum in this case appear pink and this hemolysis do not affect the results severely and most tests can be done using this serum

Server Hemolysis

The serum in this case appears reddish and this hemolysis affects the results severely and the sample must be discarded.

Effects of hemolysis

1. Hb color interferes with colometric measurement.
2. Glucose decrease due to glycolysis by RBCs enzymes
3. Bilirubin decrease due to color interference
4. Increased potassium ,LDH and GOT activity due to leakage from RBCs
5. Increased phosphorus as organic esters in the cell is hydrolyzed

Hematoma

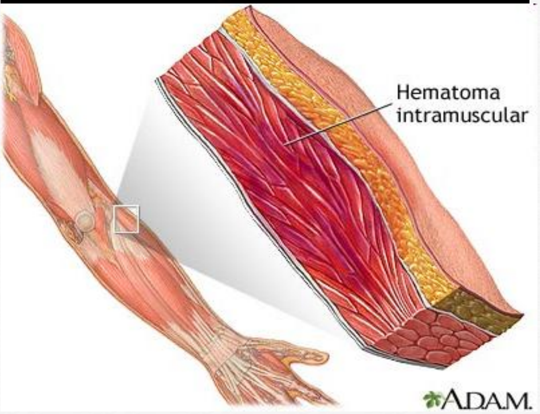
A hematoma is just a bruise like any other bruise (known as obvious bruising), and can appear even with the most skillful of phlebotomists.

Causes of hematoma

- Penetration of the vein
- Slow flow may cause hematoma
- Bending the arm after sampling
- Patients with bleeding disorders and those taking anticoagulants therapy.

How to avoid hematroma?

- Use the major superficial veins
- Don not penetrate the vein from the side
- Remove tourniquet before removing the needle
- Do not bend arm after sampling
- Apply pressure by cotton to the venipuncture site



Management of hematoma

it will go away, you can apply ice off and on after any blood-draw and use the affected arm as little as possible.

Nerve nicking

You may have a tiny branch of one of the sensory nerves of the arm actually running over the surface of the vein. Rarely the needle will hit this tiny nerve on the way into the vein. This may cause a short, sharp electric-shock type pain. This may be all that happens; however in some cases tingling type of pain may persist for one to four weeks, as the nerve heals. In this case immediately remove the needle, removing the needle immediately minimizes the chance of nerve damage.

Neonatal blood sampling (heel prick)

Purpose and indications

The **neonatal heel prick** is a common procedure for taking a blood sample from the heel of newborn which is used for a variety of tests, such as Bilirubin and TSH

Site

The recommended location for blood collection on a newborn baby or infant is the heel, only lateral parts are used not central part to avoid medial calcanous nerve, injury to this nerve can cause necrotizing osteochondritis.

Procedure

1. Make sure baby is lying in a safe position
2. Prewarming the infant's heel (for 3 to 5 minutes) is important to increases the flow of blood for collection of specimens
3. Clean the site to be punctured with warm water sponge

   Note *no alcohol to avoid hemolysis*.

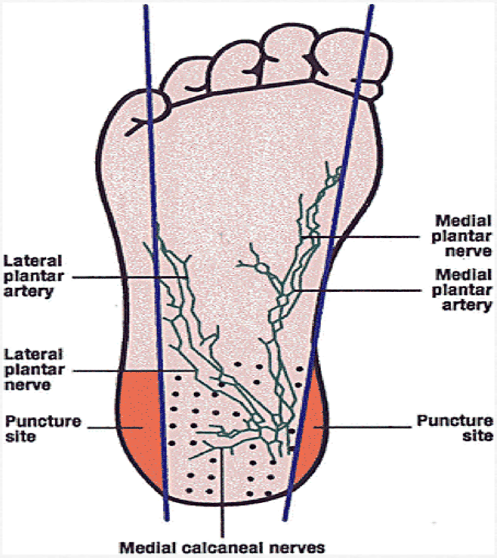
4. Dry the cleaned area with a dry cotton sponge.
5. Hold the baby's foot firmly to avoid sudden movement and to apply slight tension to the skin.

   Note *Care must be taken not to damage the heel by holding it too forcibly.*

6. Using a sterile blood lancet, puncture the heel
7. Wipe away the first drop of blood with a piece of clean, dry cotton.
8. Since newborns do not often bleed immediately, use gentle pressure to produce a rounded drop of blood.

   Note *Do not use excessive pressure because the blood may become diluted with tissue fluid.*

9. Collect blood sample using test tube, capillary tube, or automatic pipette
10. When finished, elevate the heel, place a piece of clean, dry cotton on the puncture site, and hold it in place until the bleeding has stopped.



Techniques & instruments

Spectrophotometry

What is light?

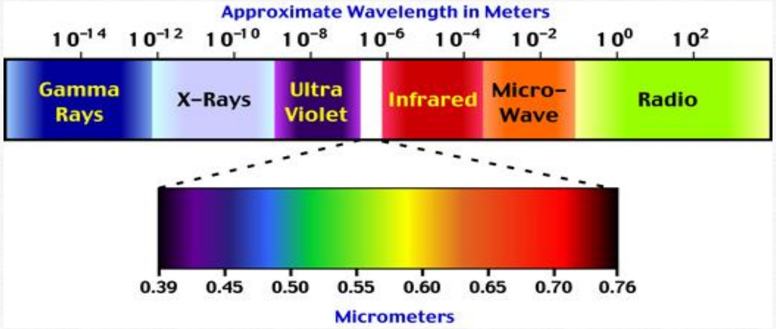
Light is a form electromagnetic radiation which exists in tiny "packets" called photons, exhibits properties of both particles and waves(photons travels in waves).

What we mean by the Wavelength?

Light have both frequency and wave length which Is the distance between peaks of the light wave and each light color is defined by its wavelength.

Different wavelengths of light appear to our eyes as different colors

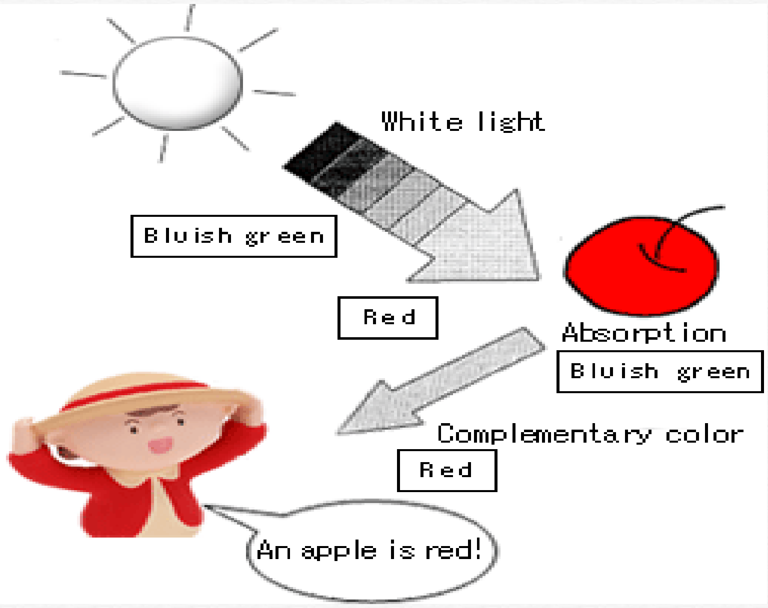
polychromatic light (White)	Monochromatic light
Light containing all wavelengths, including ultraviolet, visible light, and infrared is called white light	On the other hand, each color of light, (e.g., red, blue, etc) is called monochromatic light



The light that can be visualized by the human eye represents a narrow wavelength band (380–700 nm) between ultraviolet (UV) and infrared (IR) radiation The visible spectrum can further be subdivided according to color, often remembered by the mnemonic 'ROYGBV' standing for red, orange, yellow, green, blue and violet. Red light is at the longer wavelength end (lower energy) and violet light at the shorter wavelength end (higher energy).

Why an apple looks red to our eye?

When a certain material is exposed to light containing various colors (white light), it takes in and keeps only its favorite colors from this light (a phenomenon called "**absorption of light**"). Its disliked color (called a "**complementary color**") is then reflected, and becomes visible to our eyes as the color of the material.



In other words, an apple prefers **blue and green (490-500 nm)** and dislikes **red (610-750 nm)**. When it is exposed to white light, it will absorb blue and green, and look like red, which is the complementary color.

Wavelength (nm)	Color	Complementary color
200 - 400	Ultraviolet	-
400 - 435	Purple	Yellowish green
435 - 480	Blue	Yellow
480 - 490	Verdigris patina	Sour orange
490 - 500	Bluish green	Red
500 - 560	Green	Purplish red
560 - 580	Yellowish green	Purple
580 - 595	Yellow	Blue
595 - 610	Sour orange	Verdigris patina
610 - 750	Red	Bluish green
750 - 1000	Infrared	-

Photometric measurement

It means the use of light for measurement of the concentration of a substances in solution

Photometry

Also known as colorimetry It is defined as the use of only the visible portion of light spectrum for measurement of concentration

Spectrophotometry

It is defined as the use of the visible and UV portions of light spectrum for measurement of concentration

Absorbance and Transmittance

Absorbance

Absorbance is the measure of the quantity of light that a sample absorb (neither transmits nor reflects) and is proportional to the concentration of a substance in a solution.

The absorbance of a solution depends on:

- Nature of substance
- Concentration of substance
- Wavelength of light
- Path of light

Optical density (OD)

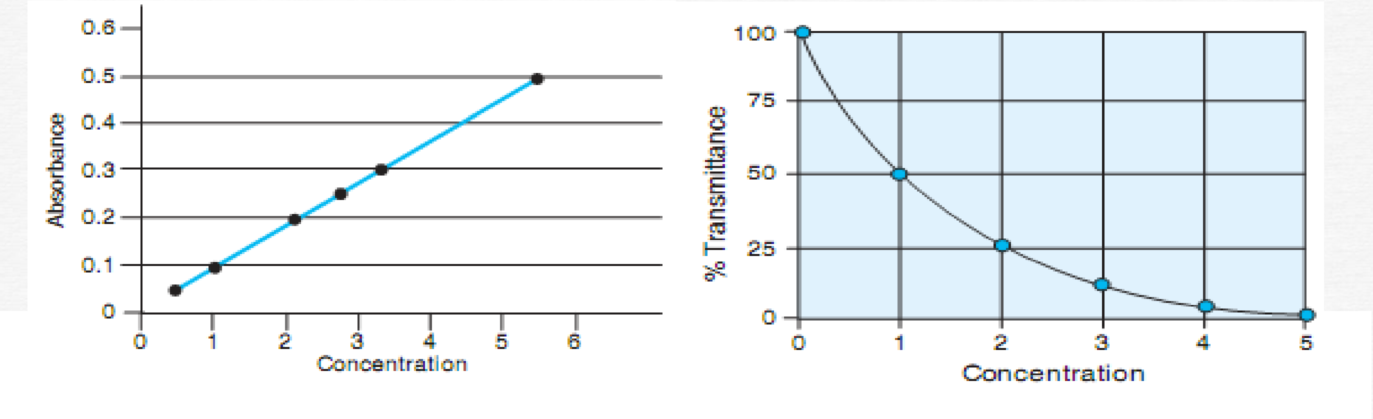
The O.D. is used the same as absorbance and directly proportional to the concentration of the colored compound.

As Concentration (C) increases, light **Absorption (A)** increases, linearly.

Transmittance

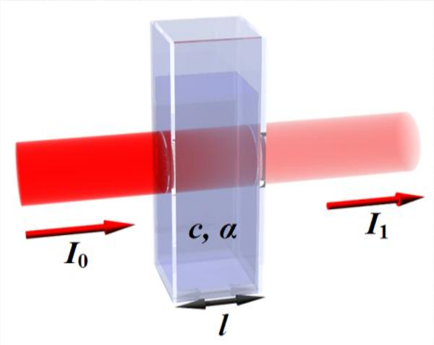
The transmittance of a sample is the ratio of the intensity of the light that has passed through the sample to the intensity of the light when it entered the sample and displayed as a percentage (%T)

As Concentration (C) increases, light **Transmission (%T)** decreases,



Relation between transmittance and absorbance

$$A = -\log T = -\log (I_1 / Io)$$



So, if all the light passes through a solution without any absorption, then absorbance is zero, and percent transmittance is 100%. If all the light is absorbed, then percent transmittance is zero, and absorption is infinite.

Law of absorbance

The Beer law

It states that the intensity of light transmitted by a colored solution decrease as the concentration increase

The absorbance of a solute is directly proportional to the concentration of a solution

$$A \propto C$$

The Lambert law

It states that the intensity of light transmitted by a colored solution decrease as the path of light increase

The absorbance of a solute is directly proportional to the path of light

$$A \propto b$$

The Beer-Lambert law

Also known as Beer's law where the two laws are usually combined together and used in all absorption analysis

$$A \propto Cb \longrightarrow A = \varepsilon Cb$$

- **A**: is the absorbance
- **C**: is the concentration of the compound in solution, expressed in mol L^{-1}
- **b**: the path length of the cuvette in which the sample is contained expressed in cm
- ε: is the molar absorbtivity with units of L mol^{-1} cm^{-1}

What we means by the molar absorbtivity?

The molar absorptivity, also known as molar extinction coefficient, is a measurement of how strongly a chemical species absorbs light at a given wavelength. It is an intrinsic property of the substance

What is the relation between the molar absorbtivity and conc.?

A compound with a high molar absorbtivity is very effective at absorbing light (of the appropriate wavelength), and hence low concentrations of a compound with a high molar absorbtivity can be easily detected.

Why we use a single wave length in photometric measurement?

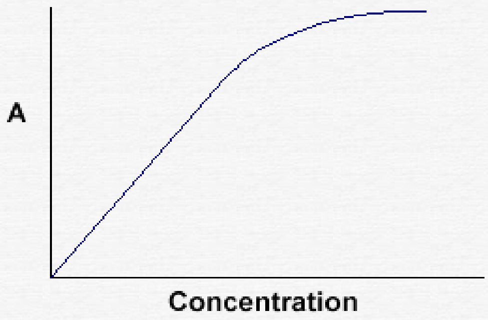
In order to obey beers low the incident radiation must be monochromatic ,each colored solution absorbs very well at specific wave length which represent the its complementary color

What we means by the Linearity?

The linearity of an analytical method is its ability to elicit test results that are directly, proportional to the concentration of analyte in samples within a given range.

The causes of nonlinearity include:

- Chemical factors: the Law is not obeyed at high concentrations due to electrostatic interactions between molecules in close proximity and scattering of light due to particulates in the sample.
- instrumental factors: non -monochromatic radiation.



How we can solve the high concentration problem that case nonlinearity?

This is can be done by making sample dilution in which a unit volume of sample of interest is combined with an appropriate volume of a solvent liquid to achieve the desired concentration.

Example: **Dilute a serum sample 1:5 dilution as follow:**

Combining 1 unit volume of serum (for example 200 μl) + 4 unit volumes of the saline (for example 800 μl) , in this case the ***dilution factor is 5*** (1 + 4 = 5 = dilution factor).

Colorimetric determination

Principle

Colorimetric analysis is a method to analyze a color. In this case a specific reagent is used to react with a specific component in the serum and a colored complex is formed which is directly proportional to the concentration of the compound in the serum.

Example:

The blood urea colorimetric procedure is a modification of the Berthelot reaction. Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. Ammonium ions react with hypochlorite and salicylate to give a green dye. The intensity of the color formed at 578 nm is proportional to the urea concentration in the sample.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2\ NH_3 + CO_2$$

$$NH_3 + \text{Salicylate} + \text{Hypochlorite} \longrightarrow \text{2,2- Dicarboxyindophenol}$$

Requirements of Colorimetric Analysis:

In colorimetric assays three tubes should be prepared as follow:

- **Reagent blank**: which contain the reagents only (without any test or standard substance) and used to set instrument at zero absorbance (Any color given by the reagents used in the analysis can be detected and eliminated)
- **Standard solution:** it is a substance identical to the test solution with a known concentration (it is different from an assay to another according to the measured parameter)
- **Test solution:** This contains the unknown concentration of the substance together with the reagents used in the test.

Note: *Sample blank can be used if sample itself is colored as in the case of bilirubin determination*

Calculations

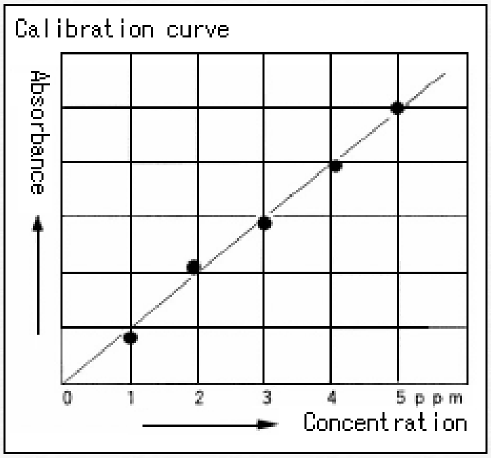
Calculation of sample concentration can be done by two methods:

Using standard concentration:

$$\text{Sample concentration} = \frac{\text{OD of test} - \text{OD blank}}{\text{OD of standard} - \text{OD blank}} \times \text{concentration of standard}$$

Using calibration curve

- Slandered calibration curve are obtained by measuring the absorbance of a series of standard solutions.
- After a series of standard solutions are analyzed a graph of absorbance versus concentration is drawn and it will be a linear relationship.
- Solutions of unknown concentration are tested for absorbance; these absorbance results are read from the curve to determine concentration.



Note: *each slandered solution should be prepared in the same way as solution being measured*

Why colorimetric method is not suitable for enzyme assays?

As the reaction proceeds with time more amount of the product is formed and since the end product inhibit the enzyme so its activity decrease and no linear curve is obtained.

What we means by the detection limit?

It is the lowest concentration or quantity of an anylate that is detected within reasonable certainty for a given analytical procedure

What we means by the analytical range?

The range of an analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined with precision, accuracy, and linearity using the method as written without modification.

What we means by the result interference?

This term describe the unwanted effect of a compound other than the analyte in question on the accuracy of measurement of the analyte for example blood hemolysis may interfere

Kinetic determination

Principle

- This type of measurement is known as ***kinetic spectrophotometry*** and usually used for determination of enzyme activity
- This method involves measuring the ability of active enzyme to catalyze, or accelerate, the rate of a chemical reaction, converting substrate to product and the enzyme it self is not consumed in the reaction.
- In the method the rate of product formation or substrate consumption is determined by a change in absorbance per minute.
    - **Multiple absorbance measurements are made**
    - **Average change in absorbance per minute is determined (A/min)**
- Substrate concentration is in excess and all other conditions in the reaction are optimal to allow the enzyme to work at its fastest speed.

Why kinetic measurement is taken only in the initial few minutes?

- In first few minutes the reaction is in Zero-order reaction and all active sites are filled with substrate.
- Also the amount of product is not increased to a level that can cause enzyme inhibition

How we know that the reaction is linear and obey Beer law?

This condition is verified when change in absorbance per minute (A/min) is constant, despite increase or decrease in absorbance.
If change in absorbance is not constant, then a variable other than enzyme activity is also present and results should not be calculated. This circumstance is often the case when enzyme activity is markedly elevated and substrate depletion occurs rapidly.

Enzyme activity can be more accurately assessed when the sample containing the enzyme is diluted and the enzyme/substrate ratio is lower.

What are the types of kinetic measurement?

Fixed time determination

as in ***serum creatinine determination***, in this case creatinine reacts with picric acid in an alkaline medium and the absorbance of the colored complex is monitored after 30 sec and then after 90 sec exactly (sample and slandered absorbance are determined by the same way) then sample concentration is calculated as follow:

$$\text{Sample concentration} = \frac{\text{Test OD2} - \text{Test OD1}}{\text{St. OD2} - \text{St. OD1}} \times \text{concentration of standard}$$

Visible kinetic determination

as in ***serum Alkaline phosphatase determination***, in this case ALP enzyme splits the phosphate group from p-nitrophenolphosphate giving the colored p-nitrophenol (yellow color) and the increase in absorbance due to yellow color formation is monitored every 1 min up to 3 min

UV kinetic determination

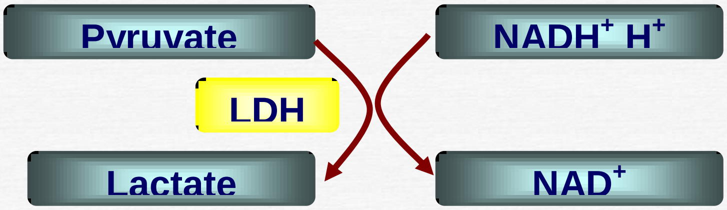
As in lactate dehydrogenase and liver Transaminase determination , in this case the enzyme reacts with the substrate but no colored product is formed instead all these reactions depends on substance that absorbs UV light such as NADH+ (NADH+ is formed or consumed during the reaction depending on the assay method) and the change in absorbance of UV light per minute is monitored every 1 min up to 3 min

How we suggested if absorbance should increase or decrease?

Simply if the substance that absorbs UV light (for example NADH+) is consumed during the reaction so the absorbance will decrease (as GOT reaction) and if the substance is formed during the reaction so the absorbance will increase.

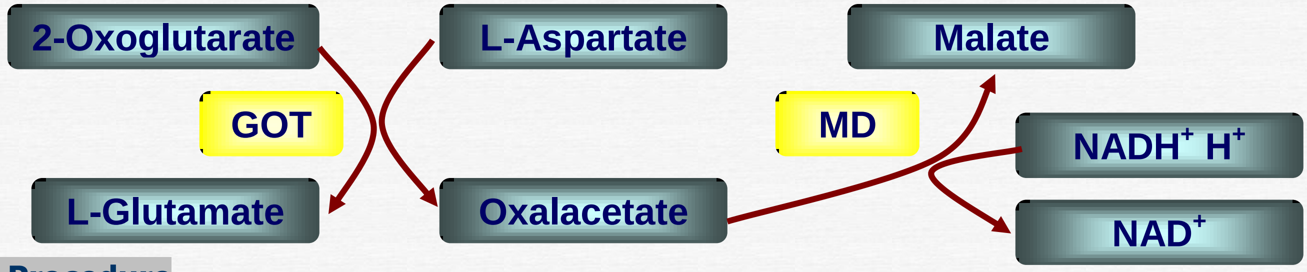
Example1: ***LDH determination***

LDH catalyzes the conversion of pyruvate to lactate. As the reaction proceeds NADH+ is oxidized to NAD+ , the disappearance of NADH+ per unit time is followed by measurement of the decrease in absorbance at 340 nm for 3 min



Example2: ***GOT determination***

GOT catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate. The oxalacetate formed in the first reaction is then reacted with $NADH^+$ in the presence of malate dehydrogenase (MDH) to form NAD^+. AST activity is determined by measuring the rate of oxidation of NADH at 340 nm.



Procedure

In kinetic assays only one tube should be prepared as follow: **Reagent + sample** , mix well and directly introduce into the spectrophotometer which measure the initial absorbance and read absorbance at 1 min interval up to 3 minutes

Calculation

- Determine the change in absorbance (ΔA340) per minute as follow:

$$\Delta A340/min = \frac{A340\ (Time\ 2) - A340\ (Time\ 1)}{Time\ 2\ (min) - Time\ 1\ (min)}$$

- Calculate the enzyme activity (IU/L)as follow:

$$\frac{\Delta A340/min \times TV \times 1000}{\varepsilon\ (mmol^{-1}cm^{-1}) \times SV \times 1.0}$$

- **TV**: *total volume in milliliter (ml)*
- **1000**: *conversion of IU/ml to IU/L*
- **ε**: *is extinction coefficient of the substance that absorbs UV*
- **SV**: *is sample volume in the reaction in milliliter (ml)*
- **1.0**: *is light path in cm*

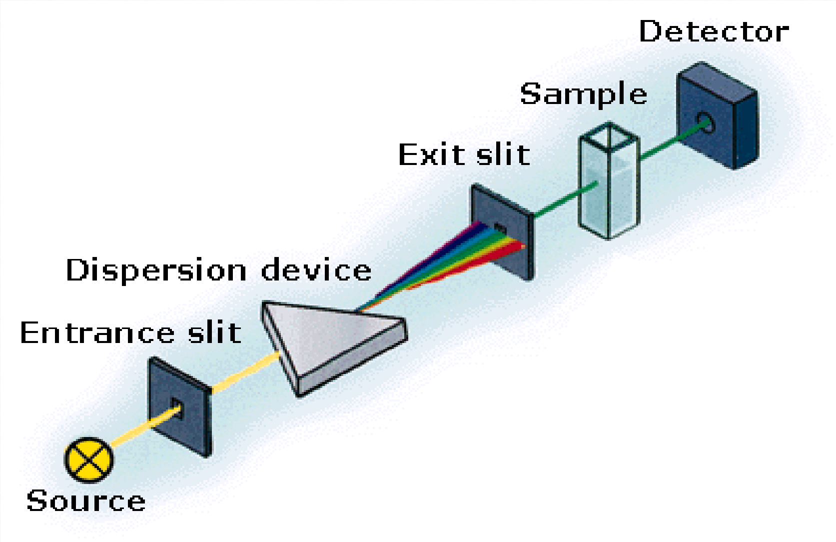
Unites: one international unit (IU) is the amount of enzyme that transforms 1 μmol of substrate per minute , in standard condition.

Spetrophotometer

Photometer (sometime known as colorimeter) and spectrophotometer are the instruments that use light in measurement.

Principles of Spectrophotometry

A spectrophotometer consists of two instruments, namely a spectrometer for producing light of any selected color (wavelength), and a photometer for measuring the intensity of light. The instruments are arranged so that liquid in a cuvette can be placed between the spectrometer beam and the photometer. The amount of light passing through the tube is measured by the photometer. The photometer delivers a voltage signal to a display device, normally a galvanometer. The signal changes as the amount of light absorbed by the liquid changes.



Basic components:

Light source

It is the source that emit radiant energy for photometric determination

In photometer it is usually tungsten lamb that emit a continuous spectrum of light in the visible range (400 - 760).

Disadvantage

- This light source produce considerable amount of heat that affect the sensitivity of photo cell
- It do not supply sufficient radiant energy for measurement below 400 nm so no UV measurement can be done in photometer

In spectrophotometer it is usually Halogen or Deuterium lamb that emit a continuous spectrum of light in the visible and UV range (198 - 1000)

Entrance slit:

This part functions in passing a very fine beam of light and passing it to the monochromator

Monochromator

It is used to isolate specific wave length of light source

In photometer optical glass filters are used to give the monochromatic light.

Filters

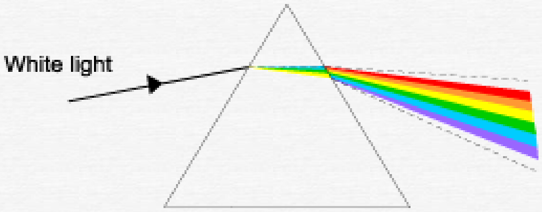
Filters act by absorbing some wavelengths and allowing a relatively broad range of wavelengths to be transmitted.

Note: *the color of filter is usually complementary to the color of solution*

In spectrophotometer prism or diffraction grating are used to give the monochromatic light of one defined wavelength.

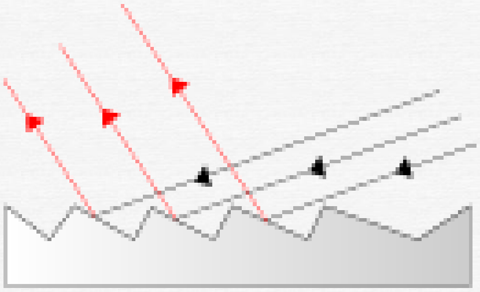
Prisms

A prism can be used to obtain monochromatic (single color) light using a slit – this is an opaque screen with a narrow opening that only allows a small amount of the spectrum to pass through.



Diffraction grating

It consist of reflecting metal or transmitting glass surfaces on which a large number of extremely fine parallel grooves have been cut. Diffraction gratings with about 13,000 grooves per cm are used for ultra-violet and visible light.



Diffraction gratings could be called 'super prisms' because they separate wavelengths very efficiently and with high intensity.

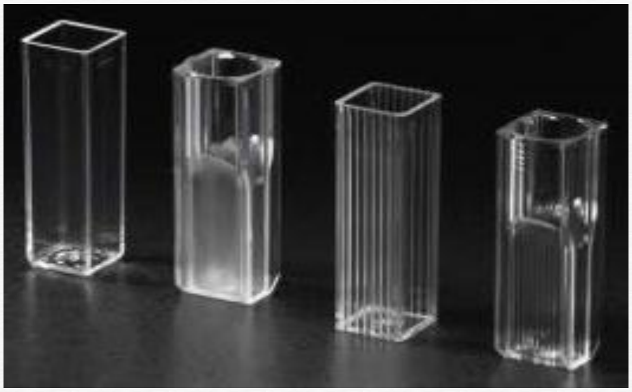
The wavelength of the light reflected from the surface of a diffraction grating depends on the angle of incidence. At each angle, the incident and reflected beams cancel each other for all but a narrow range of wavelengths (1-5 nm bandwidth compared with 20 nm for a prism). Rotation of the diffraction grating changes the wavelength of the light that is reflected away from the surface.

Exit slit

Used to isolate a narrow beam of light and improve its chromatic purity

Absorption cell (cuvets)

A cuvette is a kind of laboratory glassware, usually a small tube of circular or square cross section, sealed at one end, made of plastic, glass, or optical grade quartz and designed to hold samples for spectroscopic experiments.



Note *Square cuvete is recommended for more accurate work since the represent flat surface to light* It is usually 1cm light pass

Types

There are three different types of cuvettes commonly used, with different usable wavelengths:

- ☐ Glass, with a wavelength from 380 to 780 nm (visible spectrum)
- ☐ Plastic, with a wavelength from 380 to 780 nm (visible spectrum)
- ☐ Quartz, with a wavelength below 380 nm (ultraviolet spectrum)

Note *since light in the ultraviolet range with a wavelength of 340 nm or less hardly passes through a glass cell, so it can not be used for measurement in the UV range.*

Note *Some new plastic cuvettes (UV-transparent plastic) can be used for measurement in the UV range.*

Precautions

- Always clean the cells thoroughly and rinse at least once with a portion of the sample, before filling the sample for measurement.
- Always wipe the exposed surface of the cells dry and free from finger prints, using tissue paper.
- Clean cells thoroughly immediately after use.
- Do not leave solutions, particularly strong alkali, in the cells for periods more than an hour.
- Never use a brush or any tool for cleaning which may scratch the optical surfaces.

Flow cell

In special types of spectrophotometers known as automated analyzers a flow cell provided with electrically controlled temperature is used instead of cuvette. Because the same cuvette is in the light pass all time a source of vacuum is used to empty the flow cell and also used to introduce the sample by suction

Photodetector

The detector is a photo cell that plays a role in changing the light transmitted from a sample into an electric signal that can be detected easily. Three types of detectors are used, photoresistor, Photodiodes, and Photomultiplier tubes

Galvanometer:

This is where the electric pulses are received and converted on scale either to absorbance or to transmittance units.

Readout device

It is the screen that displays the final reading from the detector. In addition, the output may be sent to a printer, or computer.

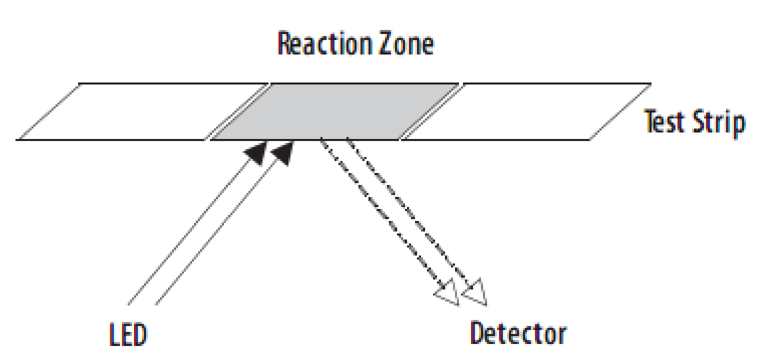
Examples of colrimeters and spectrophotometers	
	spectronic 20
	Jenway 6051
	Microlab 200
	Biosystems BTS 310
	Spectrophotometer 5010

Dry chemistry analyzer

A reflectance photometer measures the reflectance of a surface as a function of wavelength. The surface is illuminated with white light, and the reflected light is measured after passing through a monochromator. This type of measurement has many practical applications, for instance in the chemical examination of urine.

> **Note:** *simple low cost reflectance meters are available for the measurement of blood glucose*

Principle



- Reflectance photometry quantifies the intensity of a chemical or biochemical reaction generating color on a surface (e.g., test strip).
- Light is emitted at a special wavelength onto the test strip by the Instrument's light source
- The colored product absorbs that wavelength of light.
- The more analyte in the sample, the more products (color) and the less the light is reflected.
- The instrument's detector measures the reflectance of this colorimetric reaction on the test strip and converts it into an electronic signal.
- The I0 /I ratio is proportional to the reflectance value R. The reflectance measured is converted into a concentration or activity value based on test-specific standard curves.

> **Note:** *Within 2–3 minutes, test results are displayed digitally and printed from the integral printer.*

	Roche Reflotron
	Bayer automated urine Chemistry analyzer

Flame photometer

Flame photometry, more properly called flame atomic emission spectrometry, is the traditional method for determining the concentration of electrolytes in biological fluids.

A photoelectric flame photometer is a device used in chemical analysis to determine the concentration of certain metal ions, among them sodium, potassium, and lithium.

Line spectra

many metallic elements when gives sufficient energy emit this energy at wavelength characteristic for the element

Name	Color
Potassium	Lilac
Lithium	Red
Sodium	Intense yellow

How?

- Specific quantum of thermal energy is absorbed by an electron in the element atom
- Electron excitation occurs
- Electron is unstable at this high energy level so it release its excess energy as photons of certain wavelength as they change from the excited state to their ground state
- If this energy is dissipated as light , the light may consists of one or more than one energy level and therefore of different wave length
- These line spectra are characteristic for each elements

Principle of flame photometry

When a solution contain a metals as sodium , potassium or lithium aspirated into the flame, the hot flam makes:

- Evaporation of the solvent
- atomizes the metal (dissociation of chemical bonds so individual atoms is produced)
- Individual atoms is excited , (flame excites a valence electron to an upper state.)
- The electron is highly unstable in this position so light is emitted at characteristic wavelengths for each metal as the electron returns to the ground state.
- Optical filters are used to select the emission wavelength monitored for the analyte species.

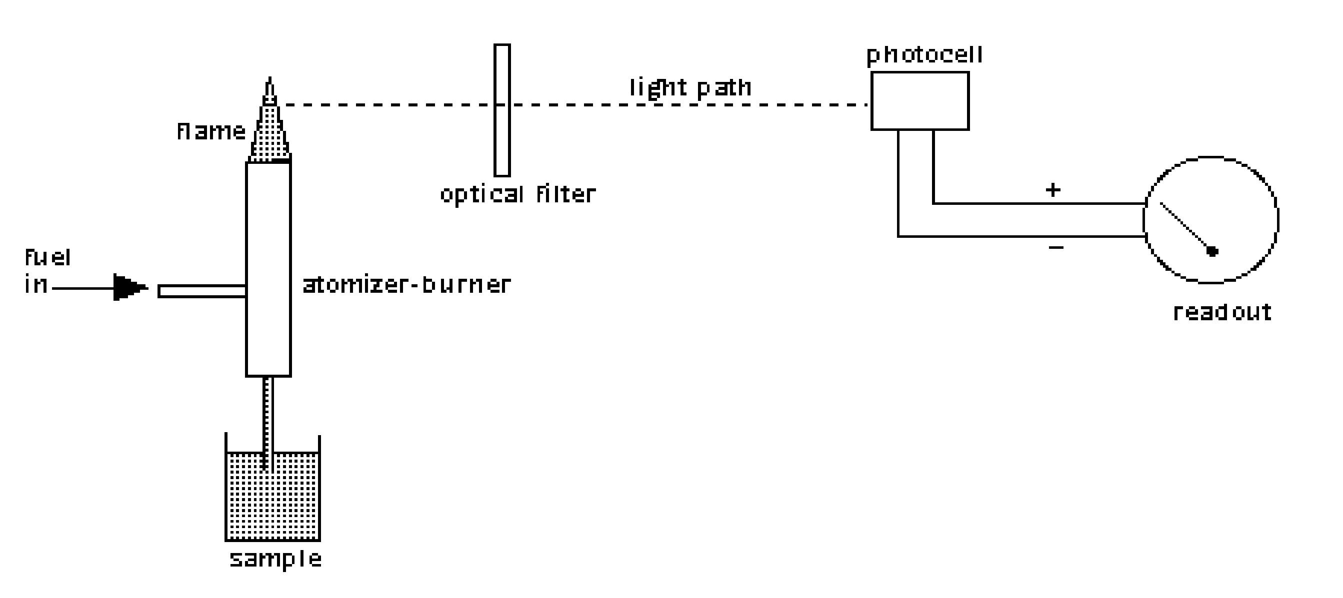
Note: *The wavelength to be used for the measurement of an element depends on the selection of a line of sufficient intensity to provide sensitivity and freedom from other interfering lines at or near the selected wavelength*

- The emitted light is detected by a detector, and is then converted to a voltage.
- Under constant and controlled conditions , the light intensity of the characteristic wavelength produced by atoms is directly proportional to the number of atoms that emit energy which directly proportional to the concentration of elements

- Comparison of emission intensities of unknowns to that of standard solutions, allows quantitative analysis of the analyte metal in the sample solution.

  Note: *Be sure to aspirate deionized-distilled water between samples to clean out the sample tube and aspirator.*

  Note: *It is important to use cleaned glassware to obtain good results.*



Advantage

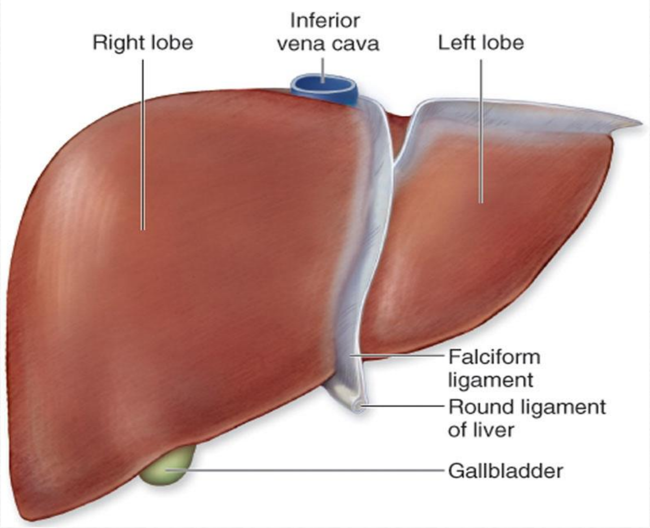
- Flame photometry is cheap compared to flame apsorption spectroscopy.
- It is a fast, simple, and sensitive analytical method that measures metals, such as Na+ and K+, that are easily excited at flame temperature.

Disadvantages,

- most of these disadvantage are related to interference and the stability of the flame and aspiration conditions, so purity, aspiration rates, solution viscosity, concomitants in the samples, will affect the measurement.

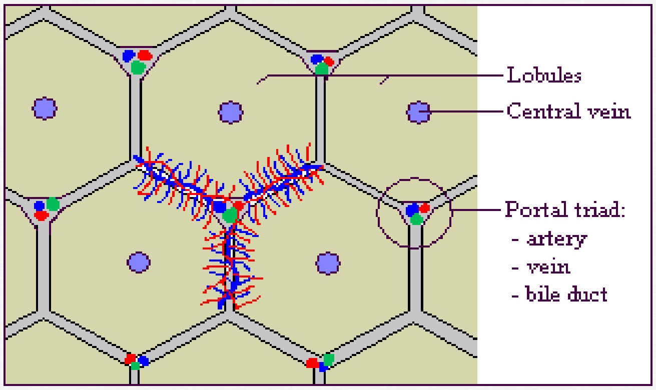
Liver function tests

Liver structure



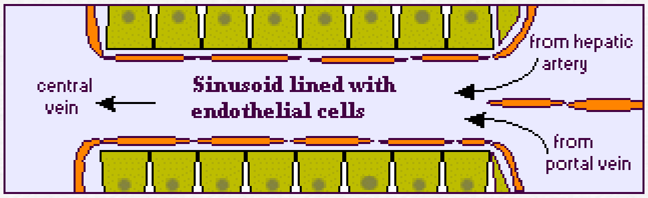
The liver is a reddish brown organ with four lobes of unequal size and shape. A human liver normally weighs 1.44–1.66 kg. It is both the largest internal organ and the largest gland in the human. It is located in the right upper quadrant of the abdominal cavity, resting just below the diaphragm. The liver lies to the right of the stomach and overlies the gallbladder. It is connected to two large blood vessels, one called the hepatic artery and one called the portal vein.

Architecture of hepatic tissue



The liver is covered with a connective tissue capsule that branches and extends throughout the substance of the liver as septae.
This connective tissue tree provides a scaffolding of support and the highway which along which afferent blood vessels, lymphatic vessels and bile ducts traverse the liver. Additionally, the sheets of connective tissue divide the parenchyma of the liver into very small units called ***lobules***.
The hepatic lobule is the structural unit of the liver. It consists of a roughly hexagonal arrangement of plates of hepatocytes radiating outward from a central vein in the center. At the vertices of the lobule are regularly distributed portal triads, containing a bile duct and a terminal branch of the hepatic artery and portal vein..

Blood flow



The liver receives a dual blood supply from the hepatic portal vein and hepatic arteries.
The hepatic portal vein carries venous blood drained from the spleen, gastrointestinal tract, and its associated organs.
The hepatic arteries carries blood from the aorta and supply arterial blood to the liver. These blood vessels subdivide into capillaries, which then lead to a lobule.
Terminal branches of the hepatic portal vein and hepatic artery empty together and mix as they enter sinusoids in the liver. Sinusoids are distensible vascular channels lined with highly fenestrated or "holey" endothelial cells and bounded circumferentially by hepatocytes.
As blood flows through the sinusoids, a considerable amount of plasma is filtered into the space between endothelium and hepatocytes (the "space of Disse.
Blood flows through the sinusoids and empties into the central vein of each lobule.
Central veins coalesce into hepatic veins, which leave the liver and empty into the vena cava.

Liver functions

Hepatocytes are metabolic super achievers in the body. They play critical roles in synthesizing molecules that are utilized elsewhere to support homeostasis, in converting molecules of one type to another, and in regulating energy balances.

- ***Carbohydrate Metabolism***
  - Glycogenesis (the formation of glycogen from glucose)
  - Glycogenolysis (the breakdown of glycogen into glucose)
  - Gluconeogenesis (the synthesis of glucose from certain amino acids, lactate or glycerol)

- ***Fat Metabolism***
  - The liver synthesizes large quantities of cholesterol, phospholipids and triglycerides
  - The liver is extremely active in oxidizing triglycerides to produce energy.
  - The liver produces and excretes bile (a yellowish liquid) required for emulsifying fats.

- ***Protein Metabolism***
  - Deamination and transamination of amino acids, followed by conversion of the non-nitrogenous part of those molecules to glucose or lipids.
  - Removal of ammonia from the body by synthesis of urea.
  - The liver produces coagulation factors such as fibrinogen and prothrombin
  - The liver is a major site of protein synthesis as albumin and globulins (except gamma globulin)

- ***Breakdown***
  - The breakdown of hormones such as insulin.
  - The liver breaks down or modifies toxic substances.

- ***Storage***
  - The liver stores a multitude of substances, including glucose (in the form of glycogen), vitamin A (1–2 years' supply), vitamin D (1–4 months' supply), vitamin B12 (1-3 years' supply), iron, and copper.

Liver Function Panel

A liver (hepatic) function panel is a blood test to check how well the liver is working. This test measures the blood levels of albumin, and liver enzymes. High or low levels may mean that liver damage or disease is present.

Why liver function tests are required?

- detect the presence of liver disease (useful in the evaluation of patients having symptoms of liver disease asfever, vomiting, abdominal pain, yellowing of your eyes or skin (jaundice), dark yellow urine, and feeling tired).
- distinguish among different types of liver disorders,
- gauge the extent of known liver damage,
- follow the response to treatment of liver disease.
- assess the effect of hepatotoxic drugs

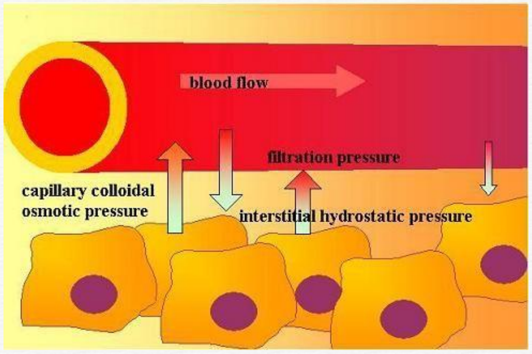
Albumin (Alb)

Overview:

Human serum albumin is the most abundant protein in human blood plasma. It is produced in the liver. Albumin comprises about half of the blood serum protein.
Albumin is synthesized in the liver as preproalbumin which has an N-terminal peptide that is removed before the nascent protein is released from the rough endoplasmic reticulum. The product, proalbumin, is in turn cleaved in the Golgi apparatus to produce the secreted albumin..

Functions of albumin

- Albumin creates 75-80% of plasma colloid oncotic pressure, which maintains vascular volume by preventing movement of fluid from the intravascular to the extravascular space.
- Transports of substances through blood as thyroid hormones, fatty acids, unconjugated bilirubin , calcium ions and drugs.



Interpretation:

Causes of hypoalbuminemia

Decreased hepatic synthesis

Liver disease

Albumin is produced exclusively in the liver. Chronic liver disease (chronic hepatitis and cirrhosis of the liver is most common cause of hypoalbuminemia.

> **Note:** *Decreased serum albumin levels are not seen in acute liver failure because it takes several weeks of impaired albumin production before the serum albumin level drops.*

> **Note:** *The serum albumin concentration is usually normal in chronic liver disease until cirrhosis and significant liver damage has occurred.*

Increased protein losses

Kidney disease

Low albumin levels can reflect diseases in which the kidneys cannot prevent albumin from leaking from the blood into the urine and being lost (as in nephrotic syndrome).

Protein-losing enteropathy

Protein losing enteropathy refers to any condition of the gastrointestinal tract that results in a net loss of protein from the body. The most common causes of protein losing enteropathy include inflammatory bowel disease and giardiasis.

Severe burns

Severe burns that damage capillaries and blood vessels cause a huge loss of serum proteins. The increased capillary permeability caused by the burn damage allows a continual leakage of serum proteins out of the vascular system.

Generalized edema

As the albumin level drops in liver disease, there is insufficient oncotic pressure to hold fluids within cells. Fluid moves into the interstitial spaces, causing generalized edema.

> **Note:** *Generally effusions are most likely to occur when albumin is < 1.5 g/dl but hypoalbuminemia in the range of 1.5 to 2.6 g/dl does not cause effusions unless accompanied by other factors that promote extravasation of fluid (e.g., high hydrostatic pressure caused by portal hypertension or renal retention of sodium).*

Causes of hyperalbuminemia

Typically this condition is a sign of severe or chronic dehydration.

> **Note:** *Albumin is not considered to be an especially useful marker of liver synthetic function; coagulation factors are much more sensitive*

> **Note:** *The prothrombin time will only be increased if the liver is so damaged that synthesis of vitamin K-dependent coagulation factors has been impaired.*

◈ Edema

Edema or Oedema , is an abnormal accumulation of fluid under the skin (swelling), or in one or more cavities of the body.
Edema is typically apparent in areas where the tissue pressure is low, such as around the eyes, and in independent tissues such ankles where hydrostatic pressure is high. Gravitational force may lead to dependent tissue edema.

Pitting edema

It can be demonstrated by applying pressure to the swollen area by depressing the skin with a finger. If the pressing causes an indentation that persists for some time after the release of the pressure, the edema is referred to as pitting edema or soft edema.

Causes of oedema

Physiological causes

- Immobility, for example either sitting or standing for long periods of time,
- During pregnancy due to altered volume/Capacitance ratio

Drugs

- The contraceptive pill
- Medication, such as corticosteroids

Pathological causes

- **Increased capillary hydrostatic pressure**
  - ***Cardiac edema***: rise in venous pressure as in congestive heart failure lead to rise in capillary hydrostatic pressure causing transudation of fluid into tissue spaces.

- **Decreased osmotic pressure**
  Due to fail of albumin below 2 g/dl or total protein below 3 g\dl as in:
  - ***Nutritional edema:*** Inadequate protein intake
  - ***Obstructive edema:*** liver disease due to decreased albumin synthesis and venous obstruction that leads to edema in the area drained by the obstructive vein
  - ***Nephrotic edema:*** renal disease due to massive albuminuria

- **Increased capillary permeability**
  - ***Inflammatory edema***: Caused by chemicals as histamine and serotonin in acute inflammation so plasma escape into tissue spaces

- **Water and sodium retention**

  Due to:
  - ***Nephritic edema:*** renal disease as glomerulonephritis
  - ***Hyperaldosteroneism:*** aldosterone increase which increase Na reabsorption with secondary retention of water

  As a result, the amount of blood circulating through your body is increased, causing the capillaries to leak even more and the surrounding tissue to swell.

- **Obstruction of lymph flow**

  Lymphedema mainly impaired the lymph flow that caused edema. The common cause of lymphedema are malignant involvement of lymph structure , removal of lymph nodes and filariasis. (this is known as non-pitting edema)

Ascites

- **What is ascites?**

  Ascites is the accumulation of fluid in the abdominal (peritoneal) cavity.

- **What causes ascites?**

  The most common cause of ascites is advanced liver disease or cirrhosis. Approximately 80% of the ascites cases are thought to be due to cirrhosis.

- **What is the mechanism of ascites?**

  Although the exact mechanism of ascites development is not completely understood, most theories suggest portal hypertension (increased pressure in the liver blood flow) as the main contributor. The basic principle is similar to the formation of edema elsewhere in the body due to an imbalance of pressure between inside the circulation (high pressure system) and outside, in this case, the abdominal cavity (low pressure space). The increase in portal blood pressure and decrease in albumin may be responsible in forming the pressure gradient and resulting in abdominal ascites.

Note: *There is also ascites formation as a result of cancers, called malignant ascites. These types of ascites are typically manifestations of advanced cancers of the organs in the abdominal cavity, such as, colon cancer, pancreatic cancer, stomach cancer, breast cancer, lymphoma, lung cancer, or ovarian cancer.*

- **What are the types of ascites?**

  A useful system has been developed based on the amount of albumin in the ascitic fluid compared to the serum albumin. This is called the Serum Ascites Albumin Gradient or SAAG.

SAAG > or = 1.1 g/dl (*Transudate*)	SAAG < 1.1 g/dl (*Exudate*)
Ascites related to portal hypertension as cirrhosis and congestive heart failure	Ascites caused by other reasons as cancer and pancreatitis

Liver enzymes (GOT,GPT)

Overview:

Alanine transaminase (ALT)

Also called Serum Glutamic Pyruvate Transaminase (SGPT) is an enzyme present in hepatocytes.

Sites

Found mainly in cytoplasm of cells.
It is widely distributed in tissues but highest levels is found in the liver

Function

It catalyzes the transfer of an amino group from alanine to a-ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate (it provide a source of pyruvate for gluconeogensis).

Aspartate transaminase (AST)

Also called Serum Glutamic Oxaloacetic Transaminase (SGOT) is similar to ALT in that it is another enzyme associated with liver parenchymal cells.

Sites

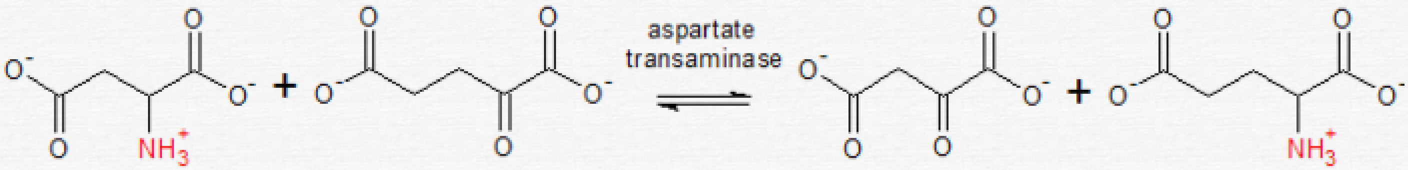
Two isoenzymes are present

- GOT1/cAST, the cytosolic isoenzyme
- GOT2/mAST, the mitochondrial isoenzyme.

It is widely distributed in tissues but highest levels is found in liver , heart , skeletal muscles and RBCs

Function

AST catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and, as such, is an important enzyme in amino acid metabolism (it provide a source of oxaloacetate for krebs cycle).



Interpretation:

Why ALT is a more specific indicator of liver damage?

The difference is that ALT is found predominantly in the liver, with clinically negligible quantities found in the kidneys, heart, and skeletal muscle, while AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells. As a result, ALT is a more specific indicator of liver damage than AST

Elevation of only GPT

ALT levels can increase in response to strenuous physical exercise. Remain elevated for about two days

Elevation of only GOT

myocardial infarction

During myocardial infarction only AST increase and ALT is normal or marginal elevated. It starts to increase ***4-6h after onset*** of infarction ***peaks16-48h*** then return to normal ***3-5 days*** later.

Note: *There duration and extent of increase being related to the size of infraction*

Note: *high value donate bad prognosis (values 10-15 times ULN considered fatal)*

Note: *GPT may also be increased in cases of severe infarction due to liveer hypoxia*

Skeletal muscle disease

Skeletal muscle trauma (AST is moderately increase
Duchenne muscular dystrophy.

Note: *AST and ALT is normal in muscle disease of neurogenic origin as mysthenia gravies.*

Note: *In muscle disease AST and CK increase but AST has a longer half life so increase in AST persists for longer than increase in CK.*

Hemolytic anemia

such as that caused by sickle cell anemia

Elevation of both GOT & GPT

Acute hepatitis

Liver enzymes are elevated in acute hepatitis due to cell necrosis and membrane damage . In this disease AST and ALT increase even before the clinical sign and symptoms of disease appear.
Very high levels of liver enzymes are usually due to acute hepatitis, often due to a virus infection.

Liver enzymes starts to rise **(30 times)** before jaundice ,peaks **(100 times) 6-10 days** after jaundice then return to normal **2-6 weeks** after jaundice

ALT is higher than AST in mild cases
AST is higher than ALT in severe cases due to mitochondrial affection

Extent of rise reflects the degree of hepatocellular damage

AST return to normal faster than ALT so AST is used to follow up

Chronic hepatitis

During chronic hepatitis

AST and ALT levels remain elevated (but not very high)

In advanced cirrhosis

The increase in liver enzymes is less marked and the liberation of enzymes from liver cells is now much reduced but failure of function is very marked (albumin and PT decreased)

- ***Liver carcinoma***

  Moderate elevation of AST and ALT is found in individuals with primary and metastatic hepatoma

  In liver carcinoma AST is usually higher than ALT

- ***Toxic hepatitis***

  Elevation of AST and ALT is found in toxic hepatitis

  - Highest level with drugs that acts on liver cells causing acute liver necrosis as paracetamol overdose
  - Moderate level with drugs that cause intra-hepatic cholestasis as oral contraceptives

- ***Alcoholic hepatitis***

  When liver damage is due to alcohol, both AST and ALT is moderately elevated

  AST often increases much more than ALT.

De-Ritis ratio

It is the ALT / AST ratio, normally less than 1

- **Elevated level :** mild liver disease
- **Decreased level :** chronic liver disease

Alkaline phosphatase (ALP)

Overview:

Sites

Alkaline phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. ALP is also present in bone , intestine and placental tissue

Functions of phosphatase

Alkaline phosphatase is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called dephosphorylation. As the name suggests, alkaline phosphatases are most effective in an alkaline environment.

Role inside the body

Its role is not fully known but its attachment to cell membrane suggesting an association between ALP activity and membrane transport so its function may be:

- Facilitate the transferee of metabolites across cell membrane
- Calcification process in bone synthesis

ALP Isoenzymes

Four isoenzymes are found liver isoenzyme , bone isoenzyme , Intestinal isoenzyme , Placental isoenzyme.

- High activity of serum ALP in infant is due to the predominance of BAP bone isoenzyme
- LAP liver isoenzyme accounts for most of the activity in healthy adults
- Placental isoenzyme present in second and third trimester so ALP is elevated during normal pregnancy

Interpretation:

Increased ALP level

Physiological increase

- **Age:** children usually have much higher ALP levels than adults because rapid bone growth is normal in children.
- **Pregnancy:** women in the third trimester of pregnancy have high ALP levels because the placenta produces ALP.
- **Bone healing:** normal healing of a bone fracture can raise ALP levels.

Pathological increase

- ***Liver disease***
  - **Liver disease without jaundice:**
    That affect the paranchymal cells as fatty liver and liver cirrhosis show only moderate or even normal ALP
  - **Liver disease with jaundice**
    - **Intra-hepatic cholestasis**
      This is due to suppression of bile flow due to hepatocellular failure as in hepatocellular carcinoma or metastatic neoplasia (cancer that has spread (metastasized) to the liver from another location in the body.).
    - **Extra-hepatic cholestasis**
      This is due to suppression of bile flow due to mechanical obstruction as gall bladder stone and pancreatic head cancer

  Note: elevation is more marked in case of extra-hepatic than in intra-hepatic and the degree of obstruction is parallel to increased elevation

- ***Bone disease***
  High ALP levels can be caused by bone diseases:
  - Paget's disease
  - osteosarcoma (so it can be used as a tumor marker for bone cancer)
  - Bone metastases of prostatic cancer (High / very high ALP values)
  - Fractured bone(active bone formation occurring as ALP is a byproduct of osteoblast activity)

- ***Due to drugs***
  - That cause cholestasis: as antidepressants and immunosuppressant
  - That cause cell toxicity: as paracitamol and antiepileptic

Decreased ALP level

- ***Cretinism***
  Hypothyroidism in infancy leads to failure of physiological and mental growth
- ***Congenital hypophsphatesia***
  The metabolic basis of hypophosphatasia stems from a molecular defect in the gene encoding tissue non-specific alkaline phosphatase (TNSALP). TNSALP is an enzyme found in the outer surface of osteoblast and chondrocyte cell membranes. This enzyme normally hydrolyzes several substances, including inorganic pyrophosphate (PPi). The enzyme defect leads to inorganic pyrophosphate (PPi) accumulates extracellularly and potently inhibits formation of hydroxyapatite (mineralization) causing rickets in infants and children and osteomalacia (soft bones) in adults.

Gamma glutamyl transpeptidase (GGT)

Overview:

Sites

gamma-glutamyl transpeptidase (also γ-glutamyltransferase, GGT, GGTP, gamma-GT) is an enzyme that transfers gamma-glutamyl functional groups. It is found in many tissues, the most notable one being the liver. Small fraction of the enzymes present in cytosol , but the larger fraction is located in the cell membrane.

Functions

It is also involved in glutathione metabolism by transferring the gamma-glutamyl moiety to a variety of acceptor molecules including water (forming glutamate), certain L-amino acids, and peptides, leaving the cysteine product to preserve intracellular homeostasis of oxidative stress.

Interpretation:

Elevated serum GGT activity can be found in diseases of the liver, biliary system, and pancreas.

Liver disease

Acute hepatitis

GGT is moderately elevated in acute hepatitis it starts to rise in the first week, peaks second week and return to normal after 3 weeks

AST and ALT are more sensitive than GGT in cases of acute hepatitis

Alcoholic liver disease (Alcoholism)

GGT is elevated by large quantities of alcohol ingestion. Alcohol may increase GGT production by inducing hepatic microsomal production, or it may cause the leakage of GGT from hepatocytes.

Isolated elevation or disproportionate elevation compared to other liver enzymes can be used to screen for chronic alcohol abuse (it will be elevated in about 75% of chronic drinkers)

GGT level is correlated with the duration of alcohol intake or dose

Hepatic malignancies

GGT is elevated in hepatic cancer and primary has higher levels than secondary.

Toxic hepatitis

Antiepileptic drugs as ***Phenobarbital*** cause GGT elevation due to that drugs stimulate excess proteins synthesis.

Biliary disease

GGT is similar to alkaline phosphatase (ALP) in detecting disease of the biliary tract (intra and extra hepatic cholestasis). Indeed, the two markers correlate well. In general, ALP is still the first test for biliary disease. The main value of GGT over ALP is in verifying that ALP elevations are, in fact, due to biliary disease.

ALP can also be increased in certain bone diseases, but GGT is not.

Pancreatic disease

GGT is moderately elevated in acute pancreatitis and pancreatic malignancy which associated with hepatobiliary obstruction.

Jaundice profile

Overview:
Bilirubin is an orange-yellow pigment found in bile. Red blood cells (RBCs) normally degrade after 120 days in the circulation. At this time, a component of the RBCs, hemoglobin breaks down into unconjugated bilirubin. Approximately 250 to 350 mg of bilirubin is produced daily in a normal, healthy adult, of which 85% is derived from damaged or old red cells that have died, with the remaining amount from the bone marrow or liver.

Metabolism of bilirubin
- Hemoglobin, which is broken down to heme as the globin parts are turned into amino acids.
- The heme is then turned into ***unconjugated bilirubin (indirect bilirubin)*** in the macrophages of the spleen.
- This unconjugated bilirubin is not soluble in water. It is then bound to albumin and sent to the liver.
- In the liver it is conjugated to glucuronic acid, forming the ***conjugated bilirubin (direct bilirubin)*** which is soluble in water.
- Much of it goes into the bile and thus out into the small intestine.
- Some of the conjugated bilirubin remains in the large intestine and is metabolized by colonic bacteria to urobilinogen, which is further metabolized to stercobilinogen, and finally oxidized to stercobilin.
- This stercobilin gives feces its brown color.
- Some of the urobilinogen is reabsorbed and excreted in the urine along with an oxidized form, urobilin.

Why Get Tested?
- It is measured in neonates to assess the severity of neonatal jaundice
- If doctor thinks that patient have signs or symptoms of liver disease, bile duct blockage, or hemolytic anemia

Normal:
Adults:
Total bilirubin 0.2 – 1.2 mg/dl
Direct bilirubin 0.0 – 0.2 mg/dl
Neonates: usually bilirubin level less than or equal 8 mg/dl is normal

Interpretation:
Jaundice, also known as ***icterus*** is a yellowish discoloration of the skin, the conjunctival membranes over the sclerae (whites of the eyes), and other mucous membranes caused by hyperbilirubinemia (increased levels of bilirubin in the blood).

> **Note:** *Typically, the concentration of bilirubin in the plasma must exceed 1.5 mg/dl, for the coloration to be easily visible.*

Causes of jaundice
Jaundice is classified into three categories, depending on which part of the physiological mechanism the pathology affects.
The three categories are:
- ***Pre-hepatic:*** ***pathology is occurring prior the liver***
- ***Hepatic:*** ***pathology is located within the liver***
- ***Post-Hepatic:*** ***pathology is located after the conjugation of bilirubin in the liver***

Pre-hepatic jaundice (Hyper-unconjugated bilirubinaemia)

Pre-hepatic jaundice is caused by anything which causes an increased rate of hemolysis (breakdown of red blood cells) producing high levels of unconjugated bilirubin such as:-

- Malaria In tropical countries lead to increased red cell lysis.
- Certain genetic diseases, such as sickle cell anemia, spherocytosis and glucose 6-phosphate dehydrogenase deficiency can lead to increased red cell lysis and therefore hemolytic jaundice.
- Transfusion reactions (ABO incompatibility)
- Erythroblastosis fetalis (Rh incompatibility)
- Crigler-Najjar syndrome (a severe disease that results from a genetic deficiency of a hepatic enzyme needed for the conjugation of bilirubin).

Hepatic jaundice (Hyper-conjugated and Hyper-unconjugated)

Hepatocellular jaundice results from injury or disease of the parenchymal cells of the liver whereby cell necrosis reduces the liver's ability to metabolise and excrete bilirubin leading to a buildup in the blood. Hepatic jaundice causes include:-

- Acute hepatitis, Viral hepatitis
- Cirrhosis (viral and chemical)
- hepatotoxicity
- alcoholic liver disease,
- primary biliary cirrhosis
- Cholangitis

Note: *conjugated bilirubin appear in urine if total bilirubin exceed 2.5 mg\dl in blood*

Post-hepatic jaundice (Hyper-conjugated bilirubinaemia)

Cholistatic jaundice, also called obstructive jaundice, is caused by an interruption to the drainage of bile in the biliary system. The most common causes are:-

- Gallstones in the common bile duct, Cholelithiasis
- Pancreatic cancer in the head of the pancreas
- Liver flukes live in the common bile duct, causing obstructive jaundice.

Note: *Patients also can present with elevated serum cholesterol, and often complain of severe itching or "pruritus"*

Laboratory Findings in the three cases

	Pre-hepatic Jaundice	Hepatic Jaundice	Post-hepatic Jaundice
Total bilirubin	Increased	Increased	Increased
Conjugated bilirubin	Normal	Normal / Decreased	Increased
Unconjugated bilirubin	Increased	Normal / Increased	Normal
Urobilinogen	Increased	Normal / Increased	Decreased / Negative
Urine Color	Normal	Dark	Dark
Stool Color	Normal	Normal	Pale
Alkaline phosphatase levels	normal	normal	increased
Alanine transferase and Aspartate transferase levels	normal	increased	normal

Neonatal jaundice

Neonatal jaundice is a yellowing of the skin and other tissues of a newborn infant. A bilirubin level of more than 5 mg/dL manifests clinical jaundice in neonates whereas in the adults 2 mg/dL would look icteric

Causes of neonatal jaundice

Causes of neonatal jaundice can be clinically classified into 2 groups:

Early onset jaundice:

Jaundice appearing in the first few days or during the first week.

The main causes are:

1. Acute hemoiysis: In this group:

- Jaundice appears at birth or during the first 24 hours
- The jaundice is commonly severe and bilirubin level rises rapidly to reach serious levels within few days.
- Anemia is evident clinically and HB level may reach below 6 gm%

Causes are:

- RH incompatibility: It is the commonest cause of hemolysis.
- ABO incompatibility: It is less common than RH incompatibility It occurs mainly in some infants group A or B born to mothers group O. It differs from RH in the following points :
  1. *The first baby may be affected.*
  2. *The disease is milder than RH and jaundice is not severe*
- G6PD deficiency: may be the cause of severe haemolysis.

2. Physiological jaundice:

It is the commonest cause of neonatal jaundice. It has the following characters:

- Jaundice appears in the 2nd or 3rd day.
- The jaundice is not severe. Serum bilirubin level usually does not exceed 15 mg% it is usually subsides within one week of onset
- Jaundice is the only clinical manifestation. General condition is fair with good activity and suckling power.
- Anemia is absent

Causes are:

Possible mechanisms involved in Physiological jaundice

- Increase bilirubin load on liver cells due to increased red blood cell destruction
- Defective hepatic uptake of bilirubin from blood plasma
- Defective billirubin conjugation due to Decreased UDPG activity

Late onset or persistent jaundice:

In this group, jaundice either appears after the first week or persists for more than 2 weeks.

Causes are:

1. Uncconjugated hyperbilirubinemia: The main causes are

a) Prolonged physiological jaundice:

Persistence of physiological jaundice for more than 2-3 weeks should suggest, congenital hypothyroidism

b) Breast milk jaundice:

In this type, jaundice usually appears on the 7th day in some breast fed infants, and it gradually increases till it reaches its peak during the third week. The jaundice is not associated with any other signs and it may persist for several weeks

A therapeutic withdrawal of breast feeding for 3 days results in rapid decline of bilirubin level over few days.

The cause of jaundice is the presence of some substances in breast milk (pregnandiol or nonesterified long chain fatty acids) which inhibit bilirubin conjugation.

c) Chronic hemolytic anemia:

Several types of chronic hemolytic anemia (as spherocytosis, sickle cell anaemia or thalassemia) may uncommonly present in the neonatal period.

2. Conjugated or mixed hyperbilirubinemia : The main causes are

- Congenital infections with one of TORCH agents
- biliary atresia
- Alpha 1 anti- antitrypsin deficiency

Phototherapy treatment of neonatal jaundice

Newborn infant undergoing phototherapy to treat neonatal jaundice. Phototherapy works through a process of isomerization that changes the bilirubin into water-soluble isomers that can be eliminated.
In phototherapy, blue light is typically used because it is more effective at breaking down bilirubin.

Bilirubin levels that may require treatment in a full-term, healthy baby	
Baby's age	Bilirubin level
Younger than 25 hours:	Evidence of jaundice
25 to 48 hours:	Greater than 15 milligrams per deciliter (mg/dl)
49 to 72 hours:	Greater than 18 mg/dl
Older than 72 hours:	Greater than 20 mg/dl

Note: *A premature or sick baby with bilirubin levels lower than those listed above may need treatment.*

Kernicterus

Kernicterus is damage to the brain centers of infants caused by increased levels of unconjugated bilirubin.

Causes

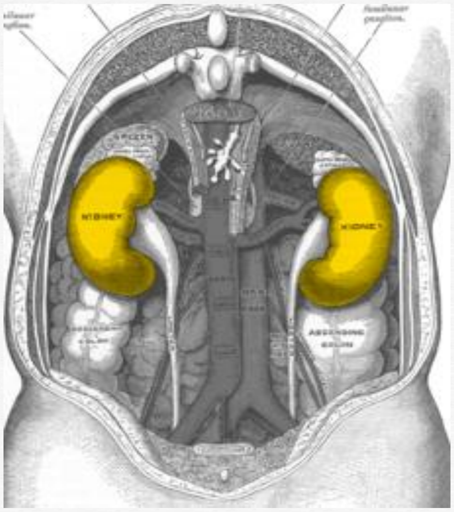
the tissues protecting the brain (the blood-brain barrier) are immature in newborns so bilirubin penetrates the brain and is deposited in cell bodies (gray matter), especially the basal ganglia, causing irreversible damage. Depending on the level of exposure, the effects range from unnoticeable to severe brain damage.

Note: *Some medications, such as the antibiotic co-trimoxazole (a combination of trimethoprim/sulfamethoxazole) may induce this disorder in the baby, either when taken by the mother or given directly to the baby, due to displacement of bilirubin from binding sites on serum albumin. The bilirubin is then free to pass into the Central Nervous System.*

Renal function tests

kidney structure

Anatomy



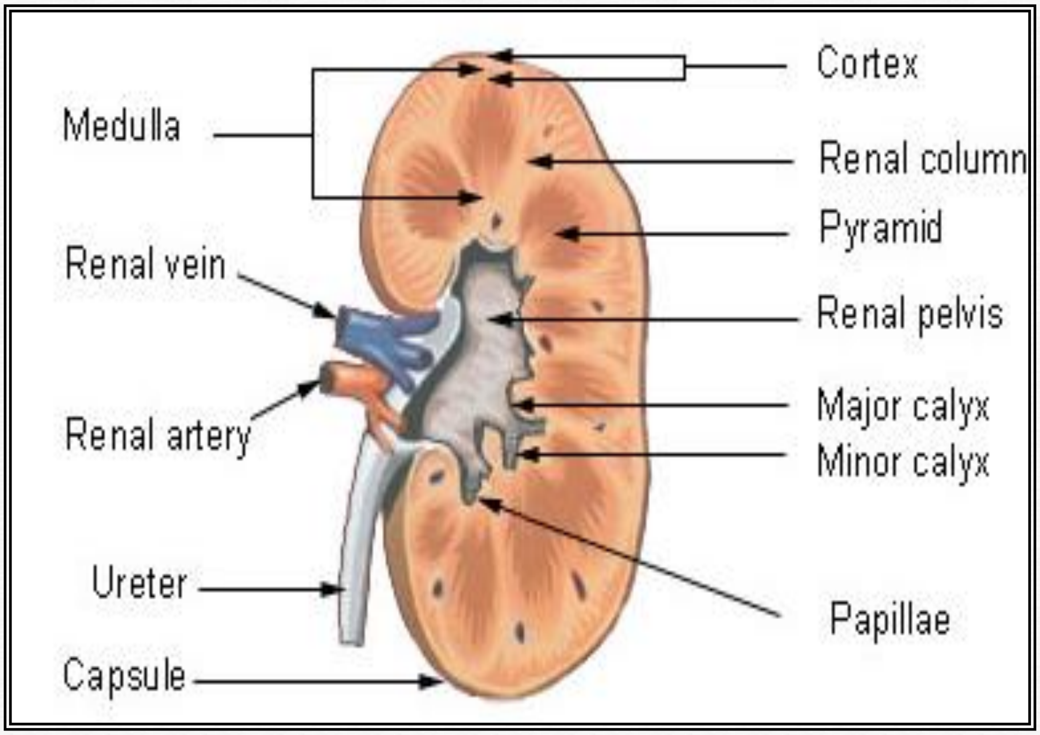
The kidneys are bean-shaped organs.
In humans, the kidneys are located in the posterior part of the abdominal cavity.
There are two, one on each side; the right kidney sits just below the diaphragm and posterior to the liver, the left below the diaphragm and posterior to the spleen.
Above each kidney there is an adrenal gland (also called the suprarenal gland)
The asymmetry within the abdominal cavity caused by the liver results in the right kidney being slightly lower than the left one

Histology

Each kidney consists of:

1- stroma

The renal capsule is a fibrous layer surrounding the kidney and covered in a thick layer of perinephric adipose tissue. It provides some protection from trauma and damage.



2- Parenchyma

It is the renal tissue and it is divided into:

Renal cortex

The renal cortex is the outer portion of the kidney between the renal capsule and the renal medulla.
It contains the renal corpuscles and the renal tubules except parts of the loop of Henle which descend into the renal medulla.

Renal medulla

The renal medulla is the innermost part of the kidney.

The renal medulla is split up into a number of sections, known as the renal pyramids.

- **Renal pyramids**

  Renal pyramids (or ***malpighian pyramids***) are cone-shaped tissues of the kidney. The renal medulla is made up of 8 to 18 of these conical subdivisions. The broad base of each pyramid faces the renal cortex, and its papilla, points internally.

Note: *The pyramids appear striped because they are formed by straight parallel segments of nephrons*

- **Renal papilla**

  In the kidney, the renal papilla is the location where the Medullary pyramids empty urine into the renal pelvis.

- **Minor calyx**

  The minor calyx, in the kidney, surrounds the apex of the malpighian pyramids. Urine formed in the kidney passes through a papilla at the apex into the minor calyx then into the major calyx.

- **Major calyx**

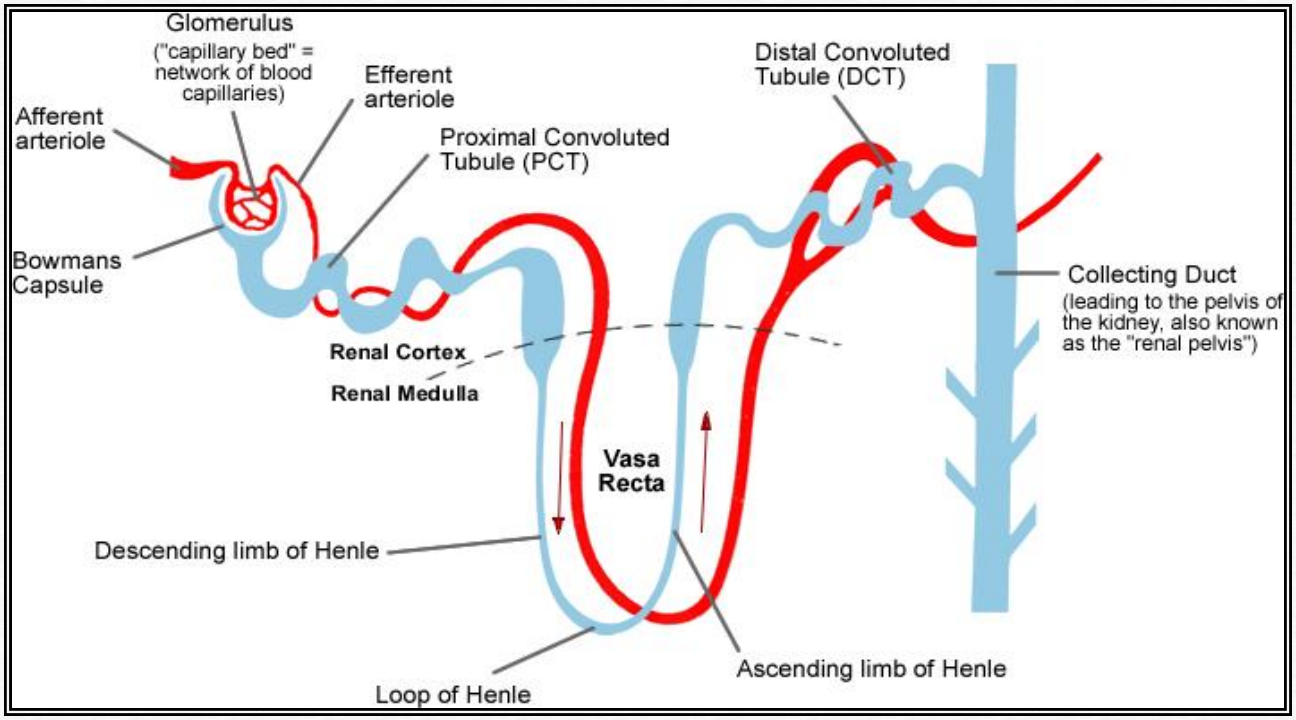
  Urine formed in the kidney passes through a papilla at the apex into a minor calyx then into major calyx before passing through the renal pelvis into the ureter.

- **Renal pelvis**

  The renal pelvis is the funnel-like dilated proximal part of the ureter in the kidney. It is the point of convergence of two or three major calyces.

Kidney Nephron

Generally nephrons are the functional units of the kidneys and there are approximately 1 million nephrons are found but they don't all function simultaneously and about 60-75% is reserve



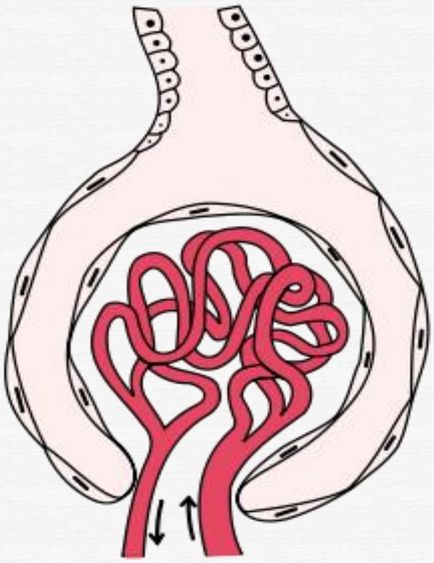
Nephrons of the kidneys are consisting of:

- **Renal Corpuscle (Malpighian corpuscle)**

It is the part of the kidney nephron in which blood plasma is filtered.

The renal corpuscle of each kidney nephron has two parts:

The Glomerulus:



Within the glomerulus there are a network of small glomerular blood capillaries that are located between the afferent arteriole bringing blood into the glomerulus and the efferent arteriole draining blood away from the glomerulus.

> **Note:** *the (outgoing) efferent arteriole has a smaller diameter than the (incoming) afferent arteriole. This difference in arteriole diameters helps to raise the blood pressure in the glomerulus.*

Glomerular Capsule:

Also known as the ***Bowman's capsule***, which is the double-walled epithelial cup within which the glomerulus is contained

The inner layer is known as *visceral layer* which contain the podocytes and the outer layer known as *parietal layer*

Bowman's space

The area between the double-walls of the Bowman's capsule is called the capsular space or Bowman's space which receive the glomerular filtrate.

Glomerular filtration barrier

The cells of the outer edges of the glomerulus form close attachments to the cells of the inner surface of the Bowman's capsule to forms a filtration membrane that enables water and solutes to pass into the capsular space:

- Any small molecules such as water, glucose, salt (NaCl), amino acids, and urea pass freely into Bowman's space
- Any proteins less than 30 kilodaltons can pass freely through the membrane.
- Cells, platelets and large proteins normally do not pass.

Functions of *filtration barrier*:

1- Prevents protein from leaking into the urine

- The slit diaphragms ensure that large macromolecules such as serum albumin and gamma globulin remain in the bloodstream while small molecules such as water, glucose, and ionic salts are able to pass.

2- Regulation of glomerular filtration rate

- When podocytes contract, they cause closure of filtration slits and this decreases the GFR by reducing the surface area available for filtration.

Renal Tubules

The renal tubule is the part of the kidney nephron into which the glomerular filtrate passes after it has reached the Bowman's capsule.

The renal tubule consists of:

- **Proximal convoluted tubule (PCT)**
  - It is the portion of the duct system of the nephron leading from Bowman's capsule to the loop of Henle.

Function

1- Absorption

The filtrate entering the proximal convoluted tubule is reabsorbed into the peritubular capillaries.

2- Secretion

- Proximal convoluted tubule is responsible for secreting organic acids, such as creatinine and other bases, into the filtrate.
- Many types of medication are secreted in the proximal tubule.

- **Loop of Henle**

  It is the portion of the nephron that leads from the proximal convoluted tubule to the distal convoluted tubule.

  The main function of this structure is to reabsorb water and ions from the urine.

- **Distal convoluted tubule (DCT)**

  The distal convoluted tubule (DCT) is a portion of kidney nephron between the loop of Henle and the collecting duct system.

Function

- It regulates pH by absorbing bicarbonate and secreting protons (H+) into the filtrate.
- Sodium and potassium levels are controlled by secreting K+ and absorbing Na+.

kidney function

Excretory function

- The kidneys produce and excrete urine, which rids the body of the end products of metabolism
- Elimination of certain foreign toxic substances and drugs before or after they are metabolized in the liver.

Regulatory function

The primary role of the kidneys is to maintain the homeostatic balance of bodily fluids by filtering and secreting metabolites and minerals from the blood.

- **Electrolyte homeostasis**

  Kidneys acts to regulate plasma volume by regulating water and electrolyte balance so any significant rise or drop in plasma osmolality is detected by the hypothalamus, which causes the pituitary gland to secrete antidiuretic hormone, resulting in water reabsorption by the kidney and an increase in urine concentration.

- **Blood pressure regulation**

  Kidney plays a role in blood pressure regulation by regulation of Sodium ions through the secretion of ***renin*** hormone which acts on ***angiotensinogen*** to produce ***angiotensin-I*** which converted in the lung to ***angiotensin-II*** that stimulate aldosterone secretion (increasing sodium reabsorption) and constrict blood vessels , this rise blood pressure by increasing the blood volume and increasing the vascular peripheral resistance.

Endocrine Function

The kidneys synthesize hormones such as:

- **Erythropoietin**

  Erythropoietin is a glycoprotein hormone produced by specialized cells in the kidneys and it regulates the production of red blood cells (RBCs) in the marrow.

- **Renin**

  Renin is produced within juxtaglomerular cells after processing and cleavage of prorenin, which is produced in the liver.

  The primary stimuli for renin release are the reduction of renal perfusion pressure and hyponatremia.

- **Active vitamin D**

  Vitamin D (cholecalciferol) can either be ingested with food or made from 7-dehdrocholesterol by the action of ultraviolet light. The liver converts cholecalciferol into 25-hydroxycholecalciferol and the proximal tubule cells of the kidney convert 25-hydroxycholecalciferol into ***1, 25-dihydroxycholecalciferol***. This is the active form of vitamin D.

Renal function tests

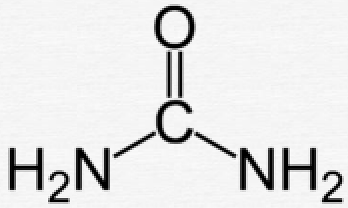
Why renal function tests are required?

- Determine whether the kidneys are functioning normally.
- Monitor the progress of kidney disease.
- Monitor the kidney function of people who take medications that can cause kidney damage.
- Monitor the effectiveness of dialysis and other treatments related to failure
- Ordered in cases of high blood pressure to assess kidney function

Blood urea

Overview

Ammonia is produced in the liver when protein is broken into its component parts (amino acids) and metabolized. The nitrogen combines with other molecules in the liver to form the waste product urea. The urea is then released into the bloodstream and carried to the kidneys, where it is filtered out of the blood and excreted in the urine. Since this is an ongoing process, there is usually a small but stable amount of urea in the blood.



The urea cycle (also known as the ornithine cycle) is a cycle of biochemical reactions occurring in many animals that produces urea (NH2)2CO from ammonia (NH3).

Sample

Serum or plasma

Note: *Do not eat a lot of meat or other protein in the 24 hours before having a blood urea nitrogen (BUN) test (fasting is recommended).*

Normal:

15 – 45 mg/dl

Note: *BUN levels in very young babies are about 2/3 of the levels found in healthy young adults, while levels in adults over 60 years of age are slightly higher than younger adults.*

Interpretation:

Azotemia is another word that refers to high levels of urea, but is used primarily when the abnormality c is not yet so severe as to produce symptoms.
Uremia is a term used to describe the illness accompanying kidney failure (also called renal failure)

Causes of high blood urea

Azotemia has three classifications, depending on its causative origin

Prerenal azotemia

Prerenal azotemia is caused by:

- Decrease in cardiac output, resulting in inadequate blood supply to the kidneys as in heart disease, dehydration, excessive blood loss that causes shock
- In this case GFR levels are decreased due to hypoperfusion, leading to a general increase in BUN level.
- It can occur from digestion of blood proteins after a GI hemorrhage
- It can occur from ingestion of high-protein diet

Renal azotemia

Renal azotemia typically leads to uremia. It is an intrinsic disease of the kidney, generally the result of renal parenchymal damage. Causes include:

- glomerulonephritis
- pyelonephritis
- Renal failure

Postrenal azotemia

Blockage of urine flow in an area below the kidneys results in postrenal azotemia. It can be caused by:

- Blockage of the ureters by kidney stones
- Pregnancy due to compression of the ureters
- Blockage of the urethra by cancer or prostatic hyperplasia

causes of low blood urea

- A low BUN value may be caused by a diet very low in protein, or malnutrition.
- Drinking excessive amounts of liquid may cause overhydration and lead to a low BUN value.
- BUN levels may normally be low in the third trimester of pregnancy.
- A low BUN value may be caused by severe liver damage

Hepatorenal syndrome

Hepatorenal syndrome is a life-threatening medical condition that consists of rapid deterioration in kidney function in individuals with cirrhosis or liver failure.
HRS is usually fatal unless a liver transplant is performed, although various treatments, including dialysis, can help.

Causes

- Hepatorenal syndrome usually affects individuals with cirrhosis and elevated pressures in the portal vein system (termed portal hypertension).
- While HRS may develop in any type of cirrhosis, it is most common in individuals with alcoholic cirrhosis,
- HRS can also occur in individuals without cirrhosis, but with acute onset of liver failure, termed fulminant hepatic failure.

Pathophysiology

- The renal failure in hepatorenal syndrome is believed to arise from abnormalities in blood vessel in the kidneys.
- Mediators released by liver disease cause dilation of blood vessels in the blood circulation which supplies the intestines
- The consequence of this phenomenon is a decrease in the "effective" volume of blood sensed by the juxtaglomerular apparatus, leading to the secretion of renin and the activation of the renin-angiotensin system
- This results in the vasoconstriction of vessels systemically and in the kidney specifically.

Serum creatinine

Overview

Creatine is synthesized in the liver, pancreas, and kidneys from the amino acids arginine, glycine, and methionine. Creatine is transported through the circulatory system to muscle where it is converted to phosphocreatine and acts as an energy reservoir much like ATP.

Creatinine is a waste product produced in your muscles from the breakdown of creatine (Creatinine is formed by the hydrolysis of creatine). Almost all creatinine is excreted by the kidneys, so blood levels are a good measure of how well your kidneys are working.

Why it is done?

Determine whether the kidneys are functioning normally.

Serum creatinine is a more specific and sensitive indicator of renal disease than BUN why?

Production of creatinine depends on an individual's muscle mass, which usually fluctuates very little. With normal kidney function, then, the amount of creatinine in the blood remains relatively constant and normal. For this reason, and because creatinine is affected very little by liver function, an elevated blood creatinine is a more sensitive indication of impaired kidney function than the BUN

Disadvantage of creatinie

Unfortunately, serum creatinine is very insensitive to even substantial declines in glomerular filtration rate. Glomerular filtration rate may be reduced by up to 50% before serum creatinine becomes elevated.

Sample

Serum or plasma

Stability in serum: Creatinine in serum is stable for twenty-four (24) hours at refrigerated temperatures (2 - 8°C) and several months when frozen (-20° C).

Normal:

Male: 0.7–1.3 mg/dl **female**: 0.6–1.1 mg/dl

> **Note:** *The quantity produced depends on the size of the person and their muscle mass. For this reason, creatinine concentrations will be slightly higher in men than in women and children.*

Interpretation:

Causes of high creatinine

Prerenal causes

- Caused by a decrease in cardiac output, resulting in inadequate blood supply to the kidneys as in heart disease, dehydration, excessive blood loss that causes shock
- Caused by Muscle disease such as acromegaly or myasthenia gravis

Renal causes

- Impaired kidney function; 50% loss of renal function is needed to increase serum creatinine from 1.0 to 2.0 mg/dL; therefore the test is not sensitive for mild to moderate renal injury
- Creatinine levels increase more slowly than blood urea nitrogen (BUN) levels, so an increase in creatinine usually indicates chronic kidney problems.

Postrenal causes

- As in postrenal azotemia

Causes of low creatinine

Low levels of creatinine are not common and are not usually a cause for concern.

Low blood creatinine levels can indicate:

- A diet very low in protein.
- A decrease in muscle mass caused by a disease, such as muscular dystrophy.
- Pregnancy can also cause low blood creatinine levels.

Total protein in urine

Overview:

The urine protein test measures the amount of protein being excreted in the urine.
The quantity of protein in a 24-hour urine sample are measured and reported as the amount of protein excreted per 24 hours.

Why it is done?

- If two or more urinalyses are positive for protein, the next step is to determine the rate of protein excretion to detect excessive protein escaping into the urine
- Evaluate and monitor kidney function, and to help detect and diagnose early kidney damage and disease (the test is the gold standard if renal function is not severely impaired)

> **Note:** *The urine protein test tells the doctor that protein is present in the urine, but it does not indicate which types are present or the cause of the proteinuria.*

Sample

The test requires the collection of all urine you pass over a 24 hour period.

> **Note:** *Drugs that may interfere with test results include Acetaminophen, Gentamicin, Nonsteroidal anti-inflammatory drugs, Penicillin G & Sulfonamides*

> **Note:** *stored urine sample must be mixed well prior analysis*

Collection method

- Ask the patient to void at the beginning of a 24-hour timed urine specimen collection.
- Discard this first specimen, and note the time.
- Mark the time the test begins and the time the collection should end on the container.

- Collect all urine voided over the next 24 hours into a large container (usually glass or polyethylene), and label it with the patient's name
- Add urine from this last voiding to the specimen in the container.
- Keep non refrigerated samples in a specified area or in the patient's bathroom.

Normal:

Healthy adults normally excrete up to150 mg/24h protein in urine daily (of that amount, less than 30 mg should be albumin).
More than 150 mg/day is defined as ***proteinuria***.

Note: *Normal in random urine is less than 10 mg/dl*

Degree of proteinuria

As kidney disease progresses, more protein enters the urine.
The following five types of proteinuria are distinguished by milligrams (mg) of protein measured during a 24-hour urine collection:

Amount of protein	Degree
30 - 300 mg/24 h	Microalbuminuria
300 — 500 mg/24 h	Mild
500 — 1000 mg/24 h	Moderate
1000 — 3000 mg/24 h	Heavy
more than 3500 mg/24 h	Nephrotic range

Interpretation:

Protein in the urine is a warning sign. It may indicate kidney damage or disease or it may be a transient elevation

Physiological proteinuria

Transient proteinuria

It is known as ***functional proteinuria*** and it is usually transient and due to a temporary increase in glomerular permeability
increased intraglomerular capillary pressure. The increased pressure favors transport of small proteins normally present in plasma through the filtration barrier.

Causes:

- Emotional stress
- Excessive muscular exertion
- Exposure to cold
- Convulsions
- Dehydration
- Sever diarrhea

Intermittent proteinuria

It is also known as ***orthostatic proteinuria*** , it is frequently associated with postural changes (protein excretion is normal when the patient is lying down but is increased when a person is sitting or standing.). Commonly occurs in young adults and the total daily excretion of protein rarely exceeds 1g/day. It is not known why orthostatic proteinuria occurs but it resolves spontaneously in about half of patients and not associated with disease (isolated proteinuria).

Pathological proteinuria

Also known as ***organic proteinuria*** or ***persistent proteinuria*** and it is more likely to reflect underlying disease

> **Note:** *In pathologic states, the level of proteinuria is rarely constant, so not every sample of urine is abnormal in patients with renal disease, and the concentration of protein in the urine is not necessarily indicative of the severity of renal disease.*

prerenal proteinuria

It is also known as ***Overflow proteinuria*** or ***overload proteinuria***, it is increased plasma levels of normal or abnormal proteins (e.g., Bence-Jones proteins, globin, myoglobin, and lysozyme) that overwhelms renal capacity to excrete these proteins (small molecular weight proteins present in the blood passing through the filtration barrier). Overflow proteinuria include hemoglobinuria, myoglobinura, Bence-Jones proteinuria.

Causes:

- Multiple myeloma
- Malignant lymphoma
- Acute pancreatitis (amylase in urine)
- Hypertension

> **Note:** *High blood pressure is the second leading cause of ESRD. Proteinuria in people with high blood pressure is an indicator of declining kidney function. If the hypertension is not controlled, the person can progress to full renal failure.*

Renal proteinuria

Glomerular proteinuria (albuminuria)

Develop when normal blood proteins are filtered by the glomeruli in varying amount depending on their molecular mass due to:

- Loss of fixed negative charge on the glomerular capillary wall
- Increased glomerular permeability leads to increased intraglomerular capillary pressure. The increased pressure favors transport of small proteins normally present in plasma through the filtration barrier.

> **Note:** *Because albumin is relatively small (65,000 daltons), it is the predominant protein lost from the glomerulus. in this case there are marked hypolbuminaemia*

Causes

- poststreptococcal glomerulonephritis
- ***nephrotic syndrome***: it is the causes of the most severe urinary protein losses.(diagnosed when protein excretion exceeds 3.5 g\day)
  It may be due to primary glomerular disease (>50% of patients) or occurs secondary to systemic lupus erythematosus(SLE) or Diabetes mellitus .It is characterized by proteinuria, hypoalbuminemia, hyperlipidemia (due to decreased Lipid catabolism and overproduction of lipoproteins) and edema (due to Lower serum oncotic pressure).

Tubular proteinuria

Develop if the tubules fail to reabsorb proteins that are normally filtered in small amounts by the glomeruli

Causes

- Nephrotoxic drugs (e.g. aminoglycosides).
- Heavy metal (e.g., lead, mercury, cadmium) Poisoning
- Acute tubular necrosis
- Renal graft rejection
- pyelonephritis

> **Note:** *Excretion of >2,000 mg/day in adults usually indicates glomerular etiology. Excretion of >3,500 mg/day points to nephrotic syndrome*

Post-renal proteinuria

Post-renal proteinuria occurs when protein enters the urine because of haemorrhage or inflammation within the lower urinary tract or genital tract.

- **Inflammation:** Inflammation and/or infection anywhere in the urogenital tract, e.g. cystitis, prostatitis, will cause proteinuria from leakage of serum protein along with leukocytes or due to increased vascular permeability.
- **Hemorrhage**: Hemorrhage frequently results in proteinuria when serum protein accompanies erythrocytes. Hemorrhage can occur anywhere within the urogenital tract (including the kidney itself) but more frequently reflects lower urinary tract (bladder disorders) or reproductive tract disease..

> **Note:** *if Post-renal proteinuria is suspected urine cytology and urine culture is recommended*

Symptoms of of proteinuria

- People who have moderate amounts of protein in their urine may have no symptoms
- Those with large amounts of protein in their urine may develop swelling around their eyes, their hands, or their feet (edema due to water retention)
- In more serious cases, the abdomen and face may swell.
- Occasionally, these people may retain enough fluid so that it goes to their lungs and causes difficulty breathing.

Complications of proteinuria

- Due to urinary losses associated with coagulation inhibitors, such as antithrombin III and protein S and C, these patients are hypercoagulable and the development of renal vein thrombosis (usually presents as acute onset of gross hematuria and back pain) in these patients may occurs

> **Note:** *nephrologists recommend the use of heparin (5000 U subcutaneously) as prophylactic anticoagulation in those patients with serum albumin levels of less than 2.5 mg/dL.*

- Patients with nephrotic syndrome are at increased risk of infection. The risk is greatest for bacterial infection (including spontaneous bacterial peritonitis) due to renal losses of immunoglobulin and complement components.

Uric acid test

Overview

In humans and higher primates, uric acid is the final oxidation (breakdown) product of purine metabolism and is excreted in urine.
Uric acid is produced by xanthine oxidase from xanthine and hypoxanthine, which in turn are produced from purine.
Purines are found in high amounts in animal internal organ food products, such as liver. A moderate amount of purine is also contained in beef, pork, fish and seafood, spinach, mushrooms, green peas, beans, and wheat germ.

Why it is done?

A uric acid blood test is done to:

- Help diagnose and monitor treatment gout.
- Check to see if kidney stones may be caused by high uric acid levels in the body.
- Check to see if medicine that increases or decreases uric acid levels is working.
- Check uric acid levels in people who are taking chemotherapy or radiation therapy

Sample

Serum or plasma

You should not eat or drink anything for 4 hours before the test.
Anticoagulants containing fluoride or citrate should not be used.
Hemolysis and lipemia should be avoided.

Normal:

Male 3.5 – 7.0 mg/dl   **Female** 2.5 – 6.0 mg/dl

Interpretation

Causes of high uric acid (*Hyperuricemia*)

Hyperuricemia is a level of uric acid in the blood that is abnormally high.

Causes of hyperuricemia can be classified into:

1) Increased production of uric acid

Primary cause:

In this case the increased purine synthesis is due to enzyme defects in pueine metabolism pathway such as:

- Lesch-Nyhan syndrome (LNS) is a rare, inherited disorder caused by a deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase
- Adenine phosphoribosyltransferase deficiency
- 5-phosphoribosyl 1-pyrophosphate synthetase overactivity

Secondary cause:

In this case hyperuricemia is secondary to increased destruction of nucleoproteins as in:

- The increased breakdown of body cells that occurs with some types of cancer (including leukemia, lymphoma, and multiple myeloma)
- Polycythemia vera
- Cancer chemotherapy (e.g., nitrogen mustards, vincristine)
- Hemolytic anemia

2) Decreased excretion of uric acid

Primary cause:

Familial juvenile gouty nephropathy : is an autosomal-dominant renal disease characterized by reduced concentrations of uric acid in the urine (hypouricuria) and increased concentrations of uric acid in the blood (hyperuricenmia) with early onset (teenage years), gout in both males and females

Secondary cause:

- ❖ *Hyperuricemia due to chronic renal failure*

  Note: *renal function test should be estimated in every case with hyperuricemia to exclude renal failure as a cause of hyperuricemia*

  Note: *there is an inappropriate rise of plasma urea and uric acid in renal failure due to increased intestinal secretion of uric acid and bacterial flora uricolysis in colon*
- ❖ *Metabollic acidosis Altered Uric acid Excretion*
  - A ketogenic diet impairs the ability of the kidney to excrete uric acid, due to competition for transport between uric acid and ketones.
  - Diabetic ketoacidosis is assioated with hyperuricemia

Causes of low uric acid (*Hypouricemia*)

Hypouricemia is a level of uric acid in blood serum that is below normal.

Note: *Hypouricemia is not a medical condition itself (i.e., it is benign), but it is a useful medical sign of a medical condition.*

Causes of Hypouricemia

- ❖ Low purine diet (Hypouricemia is common in vegetarians due to the low purine content of most vegetarian diets)
- ❖ Severe liver disease with reduced purine synthesis
- ❖ Fanconi syndrome
- ❖ Overtreatment of hyperuricemia with allopurinol or uricosuric drugs (drugs that increase the excretion of uric acid from the blood into the urine).
- ❖ cancer chemotherapy with 6-mercaptopurine or azathioprine (inhibitors of de novo purine synthesis)
- ❖ large amounts of aspirin (1,500 mg or more daily)

Note: *At low aspirin levels (as may occur in persons taking aspirin only occasionally), aspirin can increase blood uric acid due to reduction of distal tubular secretion of uric acid. On the other hand, in high doses (as may be used to treat rheumatoid arthritis), aspirin actually lowers the concentration of uric acid because it cause reduction of tubular reabsorption so increase urate excretion.*

Gout disease

- ***Overview***

  Gout is a condition that results from an overload of crystals of uric acid depositing in tissues of the body and recurring attacks of joint inflammation (arthritis).

  Note: *Gout is nine times more common in men than in women. It predominantly attacks males after puberty, with a peak age of 75. In women, gout attacks usually occur after menopause.*

  Note: *While an elevated blood level of uric acid (hyperuricemia) may indicate an increased risk of gout, the relationship between hyperuricemia and gout is unclear. Many patients with hyperuricemia do not develop gout, while some patients with repeated gout attacks have normal or low blood uric-acid levels.*

  Note: *Men with plasma uric acid concentrations exceeding 9.0 mg/dl are approximately 150 times more likely to have coexisting gouty arthritis than are men with uric acid concentrations less than 6.0 mg/dl*

- ***Causes***

Primary gout

Primary gout is associated with "essential" hyperuricemia due to metabolic overproduction of purines or underexcretion of uric acid and it occurs mainly in middle aged males

Secondary gout

Secondary gout is a complication of other medical conditions.

- Conditions that increase production of uric acid
- Conditions that decrease excretion of uric acid

- ***Risk factors***

  Risk factors for developing gout include:

  - Excessive weight gain, especially in youth,
  - Moderate to heavy alcohol intake,
  - High dietary intake of rich foods that contain purine (e.g., red meat, liver)
  - Starvation causes the body to metabolize its own (purine-rich) tissues for energy. Thus, like a high purine diet, starvation
  - High blood pressure and abnormal kidney function.
  - Patients with arteriosclerosis (serum uric acid is increased in 80% of patients with elevated serum triglycerides)
  - Medications that may interfere with the body's ability to remove uric acid (e.g., aspirin, diuretics)

In patients at risk of developing gout, certain conditions can precipitate acute attacks of gout such as:

- Dehydration
- Injury to the joint
- Fever
- Recent surgery

Symptoms

Gout usually develops in the joint of the first toe (i.e., the big toe). It also can affect the ankle or knee. Common symptoms include the following:

- Inflammation
- Pain (touching or moving the toe may be intensely painful)
- Redness
- Stiffness
- Swelling

Symptoms of gout develop quickly (sometimes in 1 day) and typically occur in only one joint at a time. Rarely, symptoms develop in two or three joints simultaneously.

Treatment

Treatment has two objectives:

- *Manage symptoms of acute attacks*

  To reduce the pain, swelling, redness, and warmth of the affected joint(s) in an acute gout attack:

  - Rest the affected joint(s).
  - Take one or more of the following medicines:
    1. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as ketoprofen
    2. Colchicine
    3. Corticosteroids

  Note: *Avoid aspirin because it may abruptly change uric acid levels in the blood and may make symptoms worse.*

- *prevent recurrent attacks*

  Take a medicine that reduces uric acid levels in the blood, which reduces the risk of future attacks such as.

  - Uricosuric agents increase elimination of uric acid by the kidneys.
  - Allopurinol decreases production of uric acid by the body.

Serum electrolytes

Serum electrolytes

an element or compound that, when melted or dissolved in water or another solvent, dissociates into ions and is able to conduct an electric current. Electrolytes differ in their concentrations in blood plasma, interstitial fluid, and cell fluid and affect the movement of substances between those compartments. Proper quantities of principal electrolytes and balance among them are critical to normal metabolism and function (eg, nerve impulse transmission, muscular contraction, water balance).

Sodium is the principal extracellular cation and potassium the principal intracellular cation. A cation is an ion with a positive charge. An anion is an ion with a negative charge.

Typically, the concentration of cations (positively charged electrolytes), eg, Na+, K+, Ca++ and Mg+, is higher in the plasma than in the interstitial fluid owing to the Donnan effect (plasma proteins have a net negative charge), whereas the anions (negatively charged), eg, Cl-, HPO4-, tend to be higher in the interstitial fluid than the plasma.

Serum potassium

Overview

Potassium is the major cation (positive ion) inside animal cells, while sodium is the major cation outside animal cells. The concentration differences of these charged particles causes a difference in electric potential between the inside and outside of cells, known as the membrane potential. The balance between potassium and sodium is maintained by ion pumps in the cell membrane.

Sodium-potassium pump (Na+/K+ ATPase)

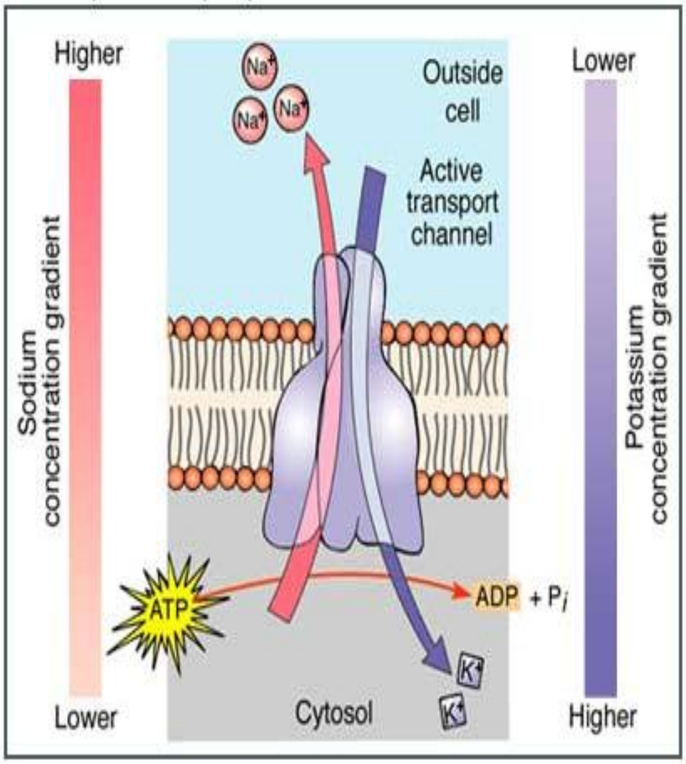
The cytosol of animal cells contains a concentration of potassium ions (K+) as much as 20 times higher than that in the extracellular fluid. Conversely, the extracellular fluid contains a concentration of sodium ions (Na+) as much as 10 times greater than that within the cell.

These concentration gradients are established by the active transport of both ions.

the transporter, Na+/K+ ATPase uses the energy from the hydrolysis of ATP to actively transport 3 Na+ ions out of the cell for each 2 K+ ions pumped into the cell.

This accomplishes several vital functions:

It helps establish a net charge across the plasma membrane with the interior of the cell being negatively charged with respect to the exterior. This resting potential prepares nerve and muscle cells for the propagation of action potentials leading to ***nerve impulses and muscle contraction***.



Why it is done?

- Monitoring acute or chronic kidney failure, that can be associated with abnormal potassium levels.
- May be ordered at regular intervals to monitor drugs that can cause kidneys to lose potassium, particularly diuretics, resulting in hypokalemia.
- Monitor the success of therapy to correct an abnormal high or low blood potassium level.
- Monitor certain cancer therapies that may cause cell lysis syndrome, a condition that occurs when cancer cells are rapidly destroyed. Cell lysis syndrome causes abnormally high blood levels of certain electrolytes, including potassium.

Normal

3.5–5.2 mEq/L

Haemolysis of samples cause falsely eleviated potassium level (Pseudohyperkalemia)

Interpretation

Hypokalemia

It refers to the condition in which the concentration of potassium (K+) in the blood is low.

Signs and symptoms

- ***Mild hypokalemia*** is often without symptoms, although it may cause a small elevation of blood pressure
- ***Moderate hypokalemia***, with serum potassium concentrations of 2.5-3 mEq/L , may cause muscular weakness, myalgia, and muscle cramps (owing to disturbed function of the skeletal muscles), and constipation (from disturbed function of smooth muscles).
- ***Severe hypokalemia*** with serum potassium concentrations less than 2 mEq/L , may cause arrhythmia and ECG abnormalities, hyporeflexia (decreased reflex response). Respiratory depression from severe impairment of skeletal muscle function is found in many patients.

Causes

- **Inadequate potassium intake**

Perhaps the most obvious cause is insufficient consumption of potassium (that is, a low-potassium diet) or starvation. However, without excessive potassium loss from the body, this is a rare cause of hypokalemia.

- **Gastrointestinal loss**

A more common cause is excessive loss of potassium, often associated with heavy fluid losses that "flush" potassium out of the body. Typically, this is a consequence of diarrhea and vomiting.

- **Urinary loss**

Certain medications can cause excess potassium loss in the urine. Diuretics, including thiazide diuretics

- **Diabetic ketoacidosis**

A special case of potassium loss occurs with diabetic ketoacidosis. In addition to urinary losses from polyuria, there is also obligate loss of potassium from kidney tubules as a cationic partner to the negatively charged ketone, β-hydroxybutyrate.

- **Alkalosis**

Alkalosis can cause transient hypokalemia by two mechanisms.

First, the alkalosis causes a shift of potassium from the plasma and interstitial fluids into cells; perhaps mediated by stimulation of Na+-H+ exchange and a subsequent activation of Na+/K+-ATPase activity.

Second, an acute rise of plasma HCO3- concentration will exceed the capacity of the renal proximal tubule to reabsorb this anion, and potassium will be excreted as an obligate cation partner to the bicarbonate.

Hyperkalemia

It refers to the condition in which the concentration of the electrolyte potassium (K+) in the blood is elevated.

❖ Signs and symptoms

Symptoms are fairly nonspecific and generally include:

malaise, palpitations , muscle weakness and in severe cases complications have developed, such as cardiac arrhythmia or sudden death.

❖ Causes

Decreased excretion (diminished potassium excretion)

- Decreased glomerular filtration rate (e.g. acute or end-stage chronic renal failure)
- ACE inhibitors or angiotensin-converting enzyme inhibitors are a group of drugs used primarily for the treatment of hypertension.
- The antibiotic trimethoprim
- Mineralocorticoid deficiency or resistance, such as: Addison's disease and Aldosterone deficiency

Increased Production (excessive endogenous potassium)

- Massive hemolysis
- Extensive burns
- Tumor Lysis Syndrome

Serum sodium

Overview

Sodium is the chief cation in fluid residing outside cells in the mammalian body (the so-called extracellular compartment), with relatively little sodium residing inside cells.

❖ Functions

- Sodium ions are necessary for, transmission of nerve impulses, heart activity, and certain metabolic functions.
- The accumulation of sodium ions outside of the cell draws water out of the cell and thus enables it to maintain osmotic balance (otherwise it would swell and burst from the inward diffusion of water).

Why it is done?

- Evaluate the water balance and electrolyte balance of the body.
- Monitor diseases of the kidneys or adrenal glands.
- Monitor treatment of certain problems, such as high blood pressure.

Normal:

135-145 mEq/L

Interpretation

Hypernatremia

is an electrolyte disturbance that is defined by an elevated sodium level in the blood

❖ **Causes**

➤ **Hypovolemic**

- Inadequate intake of water (dehydration).
- Excessive losses of water from the urinary tract, which may be caused by glycosuria.
- Water losses associated with extreme sweating.
- Severe watery diarrhea or severe vomiting.

➤ **Euvolemic**

- Excessive excretion of water from the kidneys caused by diabetes insipidus, which involves either inadequate production of the hormone, vasopressin, from the pituitary gland or impaired responsiveness of the kidneys to vasopressin.

➤ **Hypervolemic**

- Mineralcorticoid excess due to a disease state such as Cushing's Disease.

❖ **Symptoms**

Clinical manifestations of hypernatremia may include lethargy, weakness, irritability, and edema. With more severe elevations of the sodium level, seizures and coma may occur.

Hyponatremia

is an electrolyte disturbance in which the sodium concentration in the serum is lower than normal.

❖ **Causes**

➤ **Hypervolemic *hyponatremia***

excess water dilutes the sodium concentration, causing low sodium levels. Hypervolemic hyponatremia is commonly the result :

- Cirrhosis. Liver disease can cause fluids to accumulate in the body.
- Kidney problems. Kidney failure and other kidney diseases can render the body unable to efficiently remove excess fluids from the body.
- Congestive heart failure. In this condition fluid may collect in lungs (pulmonary oedema), abdomen (ascites) and legs (peripheral oedema) which in turn, reduces sodium concentration.

➤ **Euvolemic *hyponatremia***

- normal water levels are combined with low sodium levels. This condition is commonly due to chronic health conditions, or cancer.

➤ **Hypovolemic *hyponatremia***

- water and sodium levels are both low. This may occur:
- when exercising in the heat without replenishing fluid electrolytes (excessive sweating)
- with marked blood loss.
- Burns
- severe vomiting or diarrhea

- **Symptoms**

Symptoms of hyponatremia include nausea and vomiting, headache, confusion, lethargy, fatigue, appetite loss, restlessness and irritability, muscle weakness, spasms, or cramps, seizures, and decreased consciousness or coma.

The presence and severity of symptoms are associated with the level of serum sodium, with the lowest levels of serum sodium associated with the more prominent and serious symptoms.

Serum calcium

Overview:

Calcium is the most abundant mineral in the human body. The average adult body contains in total approximately 1 kg, 99% in the skeleton in the form of calcium phosphate salts. The extracellular fluid (ECF) contains approximately 22.5 mmol, of which about 9 mmol is in the serum.

Approximately 500 mmol of calcium is exchanged between bone and the ECF over a period of twenty-four hours.

Roughly half of the calcium is referred to as free or ***ionized***, and is metabolically active; the remaining half, referred to as “bound” calcium, is attached to protein and other compounds and is inactive.

Most commonly, doctors measure the total amount of calcium in the blood (called total calcium); this measures both the active and inactive forms of calcium.

- **Functions**

- Calcium plays an important role in building stronger, denser bones early in life and keeping bones strong and healthy later in life.
- Calcium (Ca^{2+}) plays an important role in signal transduction pathways, where it acts as a second messenger
- Calcium (Ca^{2+}) plays an important role in neurotransmitter release from neurons
- Calcium (Ca^{2+}) plays an important role in muscle contraction and in the electrical conduction system of the heart.
- Many enzymes require calcium ions as a cofactor, those of the blood-clotting cascade being notable examples.

Calcium regulation in the human body

Two hormones control blood calcium within a small range of values.

- ***Parathyroid hormone (PTH)*** is produced by a group of small glands in the neck (near the thyroid gland), stimulated by a decrease in ionized calcium. PTH causes the release of calcium from bone and decreases calcium losses from the kidneys, so that calcium levels rise. PTH also stimulates activation of Vitamin D by the kidneys. Vitamin D, in turn, increases calcium absorption in the intestine, but decreases calcium lost from the kidneys in urine. Overall, as Vitamin D levels rise, calcium levels rise and PTH falls.

- ***Calcitonin*** secreted from the parafollicular cells of the thyroid gland also affects calcium levels by opposing parathyroid hormone; however, its physiological significance in humans is dubious.

Normal level

The serum level of calcium is closely regulated with a normal total calcium 9-10.5 mg/dl and a normal ionized calcium of 4.5-5.6 mg/dl. The amount of total calcium varies with the level of serum albumin, a protein to which calcium is bound.

> **Note:** *Normal blood calcium values are higher in children because of their rapid growth and active bone formation. Calcium levels in children can rise to 12 mg/dL during periods of rapid bone growth.*

Corrected calcium

As about half of the total albumin in the circulation is bound to albumin, the total calcium concentration will vary with changes in albumin concentration even if the free calcium remains constant. It is possible to make an approximate correction for this effect and adjust the measured total calcium to give a value as though the albumin was normal. The usual reference intervals for calcium can then be applied.

A common equations are as follows:

Corrected Calcium (mg/dl) = Ca(measured) + (40-Alb) x 0.02

OR

Corrected Calcium (mg/dl) = (0.8 * (Normal Albumin (g/dl) - Pt's Albumin)) + Serum Ca

One can derive a corrected calcium level when the albumin is abnormal. This is to make up for the change in total calcium due to the change in albumin-bound calcium, and gives an estimate of what the calcium level would be if the albumin were within normal ranges.

> **Note:** *When there is hypoalbuminemia (a lower than normal albumin), the corrected calcium level is higher than the total calcium.*

Why it is done?

- To evaluate suspected problems with the parathyroid glands or kidneys, certain types of cancers and bone diseases
- To investigate symptoms that may be caused by a very low calcium level in the blood including muscle cramping and twitching, tingling in the fingers and around the mouth, spasms, confusion, or depression.
- To investigate symptoms that may be caused by a very high calcium level in the blood including weakness, lack of energy, poor appetite, nausea and vomiting, constipation, frequent urination, or bone pain.

> **Note:** *Newborns, especially premature and low birth weight infants, often are monitored during the first few days of life for neonatal hypocalcemia.*

Interpretation:

Hypercalcaemia

is an elevated calcium level in the blood.

Causes

- Eating large amounts of milk products or taking too many calcium-containing medications (such as antacids or vitamin supplements) can cause high blood calcium levels.
- Hyperparathyroidism
- Hyperthyroidism
- Paget's disease of the bone
- Familial hypocalciuric hypercalcaemia (defective PTH receptor)

- Hypercalcaemia of malignancy (especially breast, lung, kidney, and prostate). Cancer can cause hypercalcemia when it spreads to the bones, which releases calcium into the blood, or when cancer causes a hormone similar to PTH to increase calcium levels.
- Haematologic malignancy (multiple myeloma, lymphoma, leukaemia)

❖ **Symptoms**

- fatigue
- bone pain, especially if PTH is elevated
- kidney stones
- depression and confusion
- nausea and vomiting

Note: *Symptoms are more common at high calcium blood values (12.0 mg/dl). Severe hypercalcaemia (above 15–16 mg/dl) is considered a medical emergency: at these levels, coma and cardiac arrest can result.*

Hypocalcaemia

is the presence of low serum calcium levels in the blood, usually taken as less than 9 mg/dl or an ionized calcium level of less than 4.5 mg/dl.

❖ **Causes**

- Excessive dietary magnesium, as with supplementation.
- Hereditary hypoparathyroidism
- Acquired hypoparathyroidism
- Acute and chronic renal failure
- Defective Vitamin D metabolism
- Severe acute hyperphosphataemia
- As a complication of pancreatitis

Neonatal hypocalcemia

Newborns, especially premature and low birthweight infants, often are monitored during the first few days of life for neonatal hypocalcemia. This can occur because of an immature parathyroid gland and doesn't always cause symptoms. The condition may resolve itself or may require treatment with supplemental calcium, given orally or intravenously

❖ **Symptoms**

- Tingling or 'pins and needles' sensation in and around the mouth and lips, and in the extremities of the hands and feet. This is often the earliest symptom of hypocalcaemia.
- Muscle cramping
- Generalized tetany (unrelieved and strong contractions of the hands, and in the large muscles of the rest of the body) are seen.

Serum phosphorus

Overview:

An average adult human contains about 0.7 kg of phosphorus, about 85-90% of which is present in bones and teeth in the form of apatite, and the remainder in soft tissues and extracellular fluids (~1%). The phosphorus content increases from about 0.5 weight% in infancy to 0.65-1.1 weight% in adults.

phosphates are most commonly found in the form of adenosine phosphates, (AMP, ADP and ATP) and in DNA and RNA and can be released by the hydrolysis of ATP or ADP.

70% to 80% of the phosphates are found in bones. About 10% are found in muscle, and about 1% is in nerve tissue. The rest is found within cells throughout the body, where it is mainly used to store energy; about 1% of total body phosphorus is found within plasma (the liquid part of blood).

Biological role

- Phosphorus is a key element in all known forms of life. Inorganic phosphorus in the form of the phosphate $PO4^{3-}$ plays a major role in biological molecules such as DNA and RNA where it forms part of the structural framework of these molecules.
- Living cells also use phosphate to transport cellular energy in the form of adenosine triphosphate (ATP). Nearly every cellular process that uses energy obtains it in the form of ATP. ATP is also important for phosphorylation, a key regulatory event in cells.
- About 70% to 80% of the phosphates are combined with calcium (Calcium phosphate salts) to help form bones and teeth,
- Every cell has a membrane that separates it from its surrounding environment. Biological membranes are made from a phospholipid matrix and proteins, typically in the form of a bilayer.

Why it is done?

- Check phosphate levels if you have kidney disease or bone disease.
- Help find problems with certain glands, such as the parathyroid glands.
- Check phosphate levels if you have diabetic ketoacidosis.

Sample:

Serum sample is used

Note: *Phosphorus levels can be falsely increased by hemolysis of blood; therefore, separate serum from cells as soon as possible.*

Interpretation:

Hyperphosphatemia

Hyperphosphatemia is an electrolyte disturbance in which there is an abnormally elevated level of phosphate in the blood.

Often, calcium levels are lowered due to precipitation of phosphate with the calcium in tissues.

Causes

- **Hypoparathyroidism***:* In this situation, there are low levels of Parathyroid hormone (PTH). PTH normally inhibits renal reabsorption of phosphate, and so without enough PTH there is more reabsorption of the phosphate.
- **Chronic renal failure:** When the kidneys aren't working well, there will be increased phosphate retention.
- **Diabetic ketoacidosis** (when first seen)

Hypophosphatemia

Hypophosphatemia is an electrolyte disturbance in which there is an abnormally low level of phosphate in the blood.

Causes

- **Respiratory alkalosis*:*** Any alkalemic condition moves phosphate out of the blood into cells. This includes most common respiratory alkalemia (a higher than normal blood pH from low carbon dioxide levels in the blood), which in turn is caused by any hyperventilation (such as may result from sepsis, fever, pain, anxiety, drug withdrawal, and many other causes).
- **Alcohol abuse*:*** Alcohol impairs phosphate absorption.
- **Hyperexcretion of phosphate*:*** Phosphaturia or hyperexcretion of phosphate in the urine. This condition is divided into primary and secondary types.

  Primary hypophosphatemia is characterized by direct excess excretion of phosphate by the kidneys, as from primary renal dysfunction, and also the direct action of many diuretics.

  secondary causes, including hyperparathyroidism cause hyperexcretion of phosphate in the urine.

  Note: *Primary hypophosphatemia is the most common cause of nonnutritional rickets. Laboratory findings include low-normal serum calcium, moderately low serum phosphate, elevated serum alkaline phosphatase, hyperphosphaturia, and no evidence of hyperparathyroidism.*

- **Diabetic ketoacidosis*:*** seen after treatment due to shift of phosphorus from extracellular to intracellular space

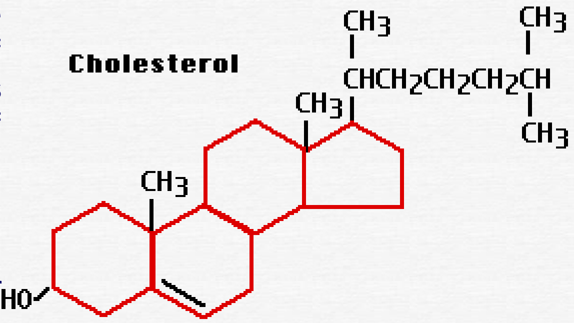
Symptoms

- Muscle dysfunction and weakness, this occurs in major muscles.
- Respiratory depression due to respiratory muscle weakness.
- Mental status changes. This may range from irritability to gross confusion, delirium, and coma.
- White cell dysfunction, causing worsening of infections.
- Instability of cell membranes due to low ATP levels: this may cause hemolytic anemia.

Lipid profile

Cholesterol

Cholesterol is small molecule, one of the steroids. The human body contains about 100 g of cholesterol. Most of this is incorporated in the membranes from which cells are constructed and is an indispensable component of them.



❖ Function

- Cholesterol is required to build and maintain membranes; it modulates membrane fluidity over the range of physiological temperatures. The hydroxyl group on cholesterol interacts with the polar head groups of the membrane phospholipids and sphingolipids, while the bulky steroid and the hydrocarbon chain are embedded in the membrane, alongside the nonpolar fatty acid chain of the other lipids. In this structural role, cholesterol reduces the permeability of the plasma membrane.
- Within cells, cholesterol is the precursor molecule in several biochemical pathways. In the liver, cholesterol is converted to bile, which is then stored in the gallbladder. Bile contains bile salts, which solubilize fats in the digestive tract and aid in the intestinal absorption of fat molecules as well as the fat-soluble vitamins, Vitamin A, Vitamin D, Vitamin E, and Vitamin K.
- Cholesterol is an important precursor molecule for the synthesis of Vitamin D and the steroid hormones, including the adrenal gland hormones cortisol and aldosterone as well as the sex hormones progesterone, estrogens, and testosterone, and their derivatives.

❖ Dietary sources

Animal fats are complex mixtures of triglycerides, with lesser amounts of phospholipids and cholesterol. As a consequence, all foods containing animal fat contain cholesterol to varying extents. Major dietary sources of cholesterol include cheese, egg yolks, beef, pork, poultry, and shrimp.

❖ Synthesis

Cholesterol synthesis occurs in the cytoplasm and microsomes (ER) from the two-carbon acetate group of acetyl-CoA.

All animal cells manufacture cholesterol with relative production rates varying by cell type and organ function. About 20–25% of total daily cholesterol production occurs in the liver; other sites of higher synthesis rates include the intestines, adrenal glands, and reproductive organs.

❖ Plasma transport

Since cholesterol is insoluble in blood, it is transported in the circulatory system within lipoproteins

❖ Regulation of cholesterol synthesis

Biosynthesis of cholesterol is directly regulated by the cholesterol levels present, though the homeostatic mechanisms involved are only partly understood. A higher intake from food leads to a net decrease in endogenous production, whereas lower intake from food has the opposite effect.

Regulation of plasma cholesterol levels via LDL receptor-mediated uptake and HDL-mediated reverse transport.

Triglycerides

A triglyceride (triacylglycerol, TAG or triacylglyceride) is an ester derived from glycerol and three fatty acids. It is the main constituent of vegetable oil and animal fats.
Triglycerides are made by the body as a storage form for unused calories taken in through diet. These lipids are stored in the cells (*adipocytes*) as fat until they are required as a source of energy through their breakdown.

High levels of triglycerides, according to the American Heart Association, are associated with the development of atherosclerosis

Lipoproteins

❖ **Importance**

In order to transport the water insoluble lipids between blood and different tissues , they are associated with ***apolipoproteins*** synthesized in the liver

❖ **Four Major Groups of Plasma Lipoproteins Have Been Identified**

Four major groups of lipoproteins have been identified that are important physiologically and in clinical diagnosis. These are:

- ***Chylomicrons:*** derived from intestinal absorption of triacylglycerol and other lipids
- ***Very low density lipoproteins:*** (VLDL), derived from the liver for the export of triacylglycerol;
- ***Low-density lipoproteins:*** (LDL), representing a final stage in the catabolism of VLDL
- ***High-density lipoproteins:*** (HDL), involved in cholesterol transport and also in VLDL and chylomicron metabolism.

❖ **Chylomicrones metabolism**

Function:

They are responsible for the transport of all dietary lipids into the circulation.

- Transfer of dietary triglycerides to the tissues mainly adipose tissue (exogenous lipids)
- Transfer cholesterol from intestine (dietary and secreted by bile) to liver

Metabolism:

- Chylomicrones are produced in the intestinal mucosal cells and contain:

(TG , CHOL , PL) + Apolipoprotein B

- It is called the ***nascent chylomicrons*** and released by the intestinal mucosa to plasma via thoracic duct.
- In plasma it receives two Apolipoproteins (APO E , APO C) from HDL
- During circulation the triglycerides core is hydrolyzed by lipoprotein lipase (which activated by APO C) to glycerol and free fatty acids.

Lipoprotein lipase

- Lipoprotein lipase is located on the walls of blood capillaries, anchored to the endothelium by chains of heparan sulfate. It has been found in heart, adipose tissue, spleen, lung, renal medulla, aorta, diaphragm, and lactating mammary gland
- Hydrolysis takes place while the lipoproteins are attached to the enzyme on the endothelium. Triacylglycerol is hydrolyzed progressively through a diacylglycerol to a monoacylglycerol and finally to free fatty acids plus glycerol.
- Some of the released free fatty acids return to the circulation, attached to albumin, but the bulk is transported into the tissue. Fatty acids originating from chylomicron triacylglycerol are delivered mainly to adipose tissue, heart, and muscle (80%), while about 20% goes to the liver.

Reaction with lipoprotein lipase results in the loss of approximately 90% of the triacylglycerol of chylomicrons

- APO C returned again to HDL and chylomicron particle begins to shrink
- This ***remnant chylomicron*** particle are removed from circulation by liver by receptor-mediated endocytosis (where liver cells recognize APO E and APO B by their receptors).
- The remnant chylomicron particle is hydrolyzed by lysosomal enzymes

> **Note:** *The clearance of chylomicrons from the blood is rapid, the half-time of disappearance being under 1 hour in humans.*

❖ VLDL metabolism

Function:

Most of the plasma VLDL are of hepatic origin. They are the vehicles of transport of triacylglycerol from the liver to the extra-hepatic tissues (endogenous lipids) .

Metabolism:

- It is produced in the liver and contain:

(TG , CHOL , PL) + Apolipoprotein B

- Then it released from liver to plasma and It is known as ***nascent VLDL***
- In plasma it receives two Apolipoproteins (APO E , APO C) from HDL
- During circulation the VLDL is hydrolyzed in tissue the same as chylomicrones by Lipoprotein Lipase casing VLDL to shrink.
- Now its called ***VLDL remenant*** (IDL)
- An exchange occurs between IDL and HDL particle where IDL take cholesterol from HDL instead of triglyceride and converted to **LDL**

❖ LDL metabolism

- Low-density lipoprotein (LDL) is the final stage of VLDL (very low-density lipoprotein) which is produced by the liver.
- The LDL contains the apolipoproteins B-100 (Apo B-100) and Apo E.
- LDL transport cholesterol to the body tissues.
- After metabolism LDL, may be taken up by the liver directly via the LDL (apo B-100, E) receptor,

It is commonly referred to as "bad cholesterol" due to the link between high LDL levels and cardiovascular disease.

❖ HDL metabolism

Function:

- A major function of HDL is to act as a repository for the apo C and apo E required in the metabolism of chylomicrons and VLDL.
- HDL is excellent acceptor of cholesterol from tissues and other lipoprotein

Because HDL can remove cholesterol from atheroma within arteries, and transport it back to the liver, they are sometimes called "good cholesterol".

Metabolism:

- HDL is synthesized and secreted from both liver and intestine.
- apo C and apo E are synthesized in the liver and transferred from liver HDL to intestinal HDL when the latter enters the plasma.
- HDL accepts cholesterol from the tissues
- once cholesterol is taken up by HDL it is esterified by enzyme ***lecithin:cholesterol acyltransferase (LCAT)***and , the cholesterol esters is very hdophopic and remain in HDL
- Finally HDL particles are taken up by the liver where CE is hydrolyzed to free cholesterol which then excreted via the bile (either as cholesterol or after conversion to bile acids)

Lipid profile

The lipid profile includes total cholesterol, HDL-cholesterol (***often called good cholesterol***), LDL-cholesterol (***often called bad cholesterol***), and triglycerides. Sometimes the report will include additional calculated values such as HDL/Cholesterol ratio or a risk score based on lipid profile results, age, sex, and other risk factors.

Why it is done?

- As part of a routine physical exam to screen for a lipid disorder.
- To check your response to medicines used to treat lipid disorders.
- To help determine risk of coronary heart disease, especially if you have other risk factors for heart disease or symptoms that suggest heart disease is present.

Sample

A blood sample after 12-hour fasting is taken

Triglycerides change dramatically in response to meals, increasing as much as 5 to10 times higher than fasting levels just a few hours after eating.

Normal:

The ***American Heart Association*** provides a set of guidelines for total (fasting) blood ***cholesterol*** levels and risk for heart disease:

Level mg/dL	Level mmol/L	Interpretation
< 200	< 5.0	Desirable level corresponding to lower risk for heart disease
200–240	5.2–6.2	Borderline high risk
> 240	> 6.2	High risk

The ***American Heart Association*** has set guidelines for ***triglyceride*** levels:

Level mg/dL	Level mmol/L	Interpretation
<150	<1.69	Normal range, lowest risk
150-199	1.70-2.25	Borderline high
200-498	2.25-5.63	High
>500	>5.65	Very high, increased risk

The ***American Heart Association*** has set guidelines for ***HDL*** levels:

Level mg/dL	Level mmol/L	Interpretation
<40	<1.03	Low HDL cholesterol, heightened risk for heart disease, <50 is the value for women
40-59	1.03-1.52	Medium HDL level
>60	>1.55	High HDL level, optimal condition considered protective against heart disease

The ***American Heart Association*** has set guidelines for ***LDL*** levels:

Level mg/dL	Level mmol/L	Interpretation
<100	<2.6	Optimal LDL cholesterol, corresponding to reduced, but not zero, risk for heart disease
100 to 129	2.6 to 3.3	Near optimal LDL level
130 to 159	3.3 to 4.1	Borderline high LDL level
160 to 189	4.1 to 4.9	High LDL level
>190	>4.9	Very high LDL level, corresponding to highest increased risk of heart disease

Calculations:

❖ LDL estimation

LDL is estimated using the ***Friedewald formula***: estimated

LDL = [total cholesterol] − [total HDL] − [estimated VLDL]. VLDL can be calculated by dividing total triglycerides by 5.

Note: *Direct LDL measures are used when triglycerides exceed 400 mg/dl. The estimated VLDL and LDL have more error when triglycerides are above 400 mg/dl*

❖ cardiac risk ratios

The cardiac risk ratio provides more information than does either value alone. The higher the ratio, the greater the risk for developing atherosclerosis.

cardiac risk ratio	Interpretation
Total cholesterol / HDL cholesterol	*(Low risk 3.3- 3.9)* *(Average risk 3.9-5.0)* *(Moderate risk 5.0-9.6)* *(High risk 9.6 -23.4)*
LDL cholesterol / HDL cholesterol	*(Low risk 0.5-3.0)* *(Moderate risk 3.0-6.0)* *(High risk >6.0)*

Interpretation:

Cholesterol abnormalities

Hypercholesterolemia

Primary hypercholesterolemia

Familial hypercholesterolemia (FH) is an autosomal dominant disorder that causes severe elevations in total cholesterol and low-density lipoprotein cholesterol (LDLc) , its due to LDL receptor defect.

Serum cholesterol levels may exceeds 1000 mg/dl

Secondary hypercholesterolemia

Elevated cholesterol levels occur in the following conditions:

- Cholestasis
- Hepatocellular disease
- Nephrotic syndrome
- Chronic renal failure
- Hypothyroidism
- Pancreatic and prostatic malignant neoplasms
- Poorly controlled diabetes mellitus
- Alcoholism
- Obesity

Elevated cholesterol levels are treated with a strict diet consisting of low-saturated fat, trans-fat free, low cholesterol foods,[38][39] often followed by one of various hypolipidemic agents.

Hypocholesterolemia

Abnormally low levels of cholesterol are termed hypocholesterolemia.
In general, the low cholesterol levels seem to be a consequence of an underlying illness, rather than a cause.
Low cholesterol (less than 100 mg/dl) is often seen when there is an existing problem like:

- Severe hepatocellular disease
- Myeloproliferative diseases
- Hyperthyroidism
- Malabsorption syndrome
- Megaloblastic anemia
- Severe burns and inflammations

Triglyceride abnormalities

Hypertriglyceridemia

Primary Hypertriglyceridemia:

Familial hypertriglyceridemia is a common disorder passed down through families in which the level of triglycerides in a person's blood are higher than normal. (Persistent very high TG (>1,000 mg/dl))
Familial hypertriglyceridemia is caused by a genetic defect, which is passed on in an autosomal dominant fashion.
Obesity, hyperglycemia, and high levels of insulin are often also present and may cause even higher triglyceride levels.

The condition is not associated with a significant increase in cholesterol levels.

Some people with this condition also have high levels of very low density lipoprotein (VLDL).

Note: *When triglycerides are very high (greater than 1000 mg/dl, there is a risk of developing pancreatitis. Treatment to lower triglycerides should be started as soon as possible.*

Secondary Hypertriglyceridemia

Elevated triglycerides levels occur in the following conditions:

- Diet low in protein and high in carbohydrates
- Hypothyroidism
- Nephrotic syndrome
- Pancreatitis
- Poorly controlled diabetes
- Liver disease, alcoholism (can be extremely high with alcoholism)
- Myocardial infarction (elevated levels may persist for several months)

Hypotriglyceridemia

There are 5 causes of low triglyceride level in body described below:

- Low fat diet
- Hyperthyroidism
- Malabsorption Syndrome
- Chronic liver disease
- Chronic inflammation of pancreas (pancreatitis)

Disorders of the Plasma Lipoproteins (Dyslipoproteinemias)

Lipoprotein disorder: Abnormal lipoprotein use or processing by the body. The main repercussions of the disorder tends to be fatty deposits called xanthomas and premature heart disease.

Primary Dyslipoproteinemias:

Several Inherited genetic defects exist that result in defects in lipoprotein metabolism lead to the primary condition of either hypo- or hyperlipoproteinemia.

Hyperlipoproteinemia

Familial hyperchylomicronemia

deficiency of LPL leads to slow chylomicron clearance, reduced LDL and HDL levels; no increased risk of coronary artery disease.

Familial dysbetalipoproteinemia

hepatic remnant clearance impaired due to apoE abnormality causes xanthomas, hypercholesterolemia and athersclerosis in peripheral and coronary arteries due to elevated levels of chylomicrons and VLDL

Familial hyperalphalipoproteinemia

increased level of HDL. It is a rare condition that is beneficial for health and longevity

Familial hyperbetalipoproteinemia

increased LDL production and delayed clearance of triacylglycerols and fatty acids. It is strongly associated with increased risk of coronary artery disease

Wolman disease

defect in lysosomal cholesteryl ester hydrolase; affects metabolism of LDL. It cause reduced LDL clearance leads to hypercholesterolemia, resulting in athersclerosis and coronary artery disease

Hypolipoproteinemia

Abetalipoproteinemia

It is a rare defect characterized by no chylomicrons, VLDLs or LDLs due to defect in apoB expression. It cause intestine and liver accumulate, malabsorption of fat, retinitis pigmentosa, erythrocytes have thorny appearance

Familial hypobetalipoproteinemia

LDL concentrations 10-20% of normal, VLDL slightly lower, HDL normal. It cause mild or no pathological changes

Familial hypoalphalipoproteinemia

HDL cholesterol < 35 mg/dl leads to premature atherosclerosis

Tangier disease

Reduced HDL concentrations, no effect on chylomicron or VLDL production. There is a tendency to hypertriacylglycerolemia; some elevation in VLDL.

Secondary Dyslipoproteinemias:

The recognition of secondary causes of dyslipoproteinemias is important such as:

- Obesity, and high fat diet contribute to dyslipoproteinemia.
- Diabetes is the main metabolic disorders causing dyslipoproteinemias. In this case a resistance of the peripheral tissue to insulin exists and this contributes predominantly to the increase in lipids in the blood.
- Estrogens may cause an increase of triglycerides and HDL cholesterol.
- An acute glomerulonephritis can cause an extensive increase in LDL cholesterol
- Chronic renal failure is associated with decreased HDL cholesterol.
- The most important drug-induced dyslipoproteinemias associated with premature atherosclerosis are induced by corticosteroids, immune-suppressant drugs, and protease inhibitors used as antiviral therapy in HIV-infected patients.

Atherosclerosis

Arteriosclerosis is a general term describing any hardening (and loss of elasticity) of medium or large arteries (from the Greek ***Arterio***, meaning artery, and ***sclerosis***, meaning hardening)
Atherosclerosis is the most common form of arteriosclerosis which is a hardening of an artery specifically due to an atheromatous plaque

Cholesterol is strongly implicated in the development of atherosclerosis: fatty deposits (plaques) that form on the inside of blood vessels and predispose to heart attacks.

Signs and symptoms

Atherosclerosis typically begins in early adolescence, and is usually found in most major arteries, yet is asymptomatic and not detected by most diagnostic methods during life.

For about 65% of men and 47% of women, the first symptom of atherosclerotic cardiovascular disease is heart attack

Risk factors include:

- Tobacco smoking, increases risk by 200% after several pack years
- Age (you are a male 45 years or older or a female 55 years or older)
- Hypertension (Blood pressure of 140/90 or higher),increasing risk by 60%
- Having diabetes or Impaired glucose tolerance (IGT)
- Low HDL (less than 40 mg/dl)
- High serum concentration of low-density lipoprotein (LDL)
- Elevated plasma cholesterol levels are believed to be a major factor in promoting atherosclerosis
- It is now recognized that high triacylglycerols are an independent risk factor.
- Obesity is also a risk factor.
- Stress (occupations with high stress index are known to have susceptibility for atherosclerosis)

Mechanism

- Atherosclerosis develops from low-density lipoprotein molecules (LDL) becoming oxidized by free radicals, particularly reactive oxygen species (ROS).
- When oxidized LDL comes in contact with an artery wall, a series of reactions occur to repair the damage to the artery wall caused by oxidized LDL
- The body's immune system responds to the damage to the artery wall caused by oxidized LDL by sending macrophages and T-lymphocytes to absorb the oxidized-LDL forming specialized foam cells.
- Unfortunately, these white blood cells are not able to process the oxidized-LDL, and ultimately grow then rupture, depositing a greater amount of oxidized cholesterol into the artery wall. This triggers more white blood cells, continuing the cycle.

Cardiac function tests

human heart

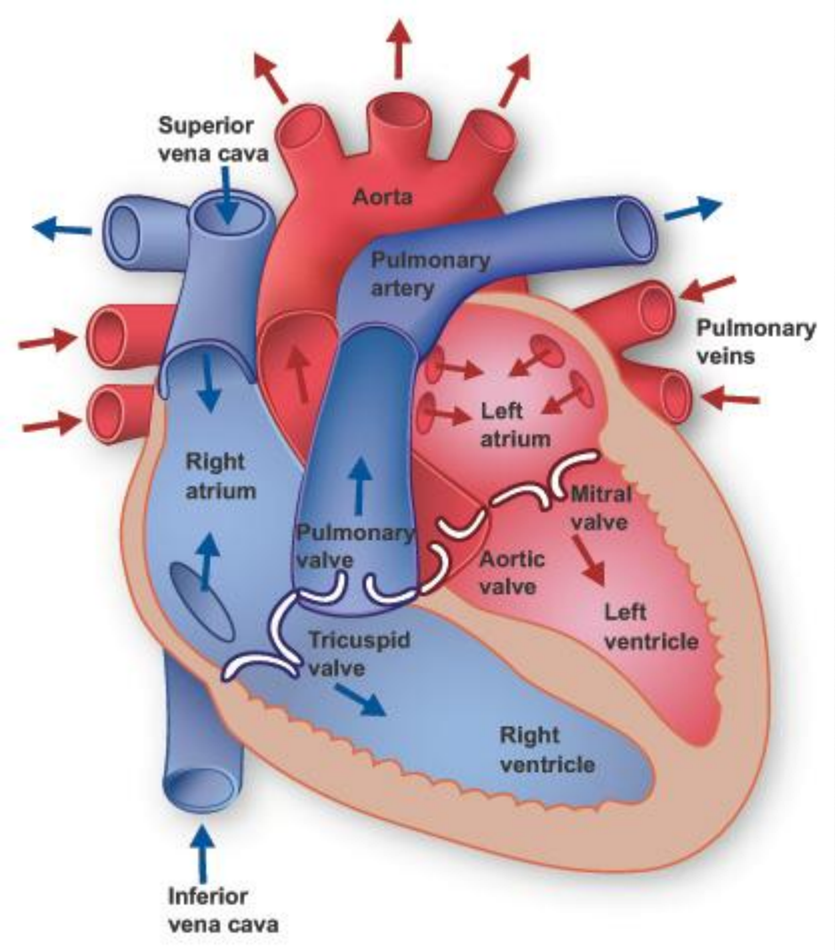
The human heart provides a continuous blood circulation through the cardiac cycle and is one of the most vital organs in the human body.

Heart Wall

The heart is located between lungs in the middle of chest, behind and slightly to the left of sternum. A double-layered membrane called the pericardium surrounds your heart like a sac. The outer layer of the pericardium (***Epicardium***) surrounds the roots of your heart's major blood vessels and is attached by ligaments to your spinal column, diaphragm, and other parts of your body. The inner layer of the pericardium (***Endocardium***) is attached to the heart muscle.

Heart Chambers

The heart is divided by a septum into two halves, and the halves are in turn divided into four chambers.
The upper chambers are called the left and right atria, and the lower chambers are called the left and right ventricles. The left ventricle is the largest and strongest chamber in heart. The left ventricle's chamber walls are only about a half-inch thick, but they have enough force to push blood through the aortic valve and into your body.



Heart Valves

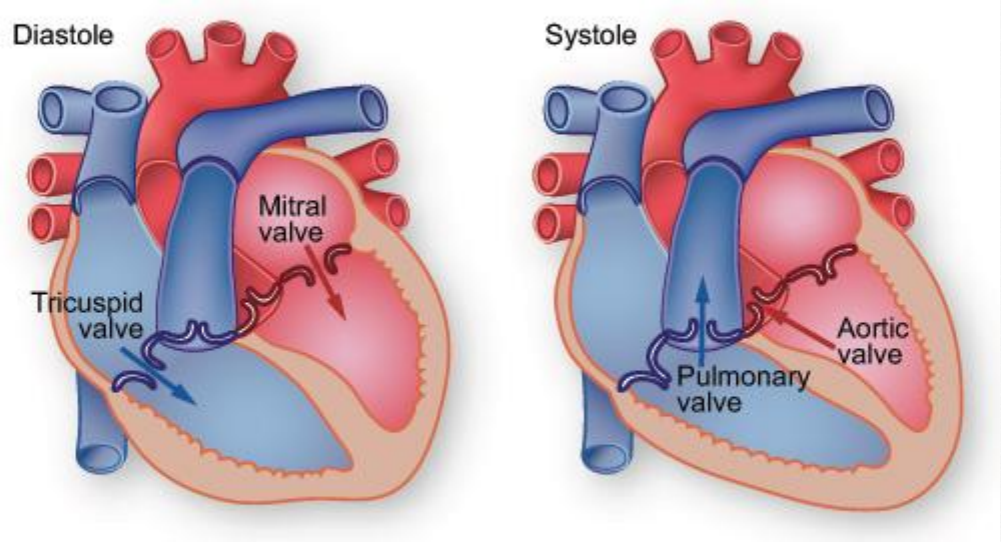
Heart valves are flap-like structures that allow blood to flow in one direction. *Four types of valves regulate blood flow through your heart:*

The tricuspid valve regulates blood flow between the right atrium and right ventricle.

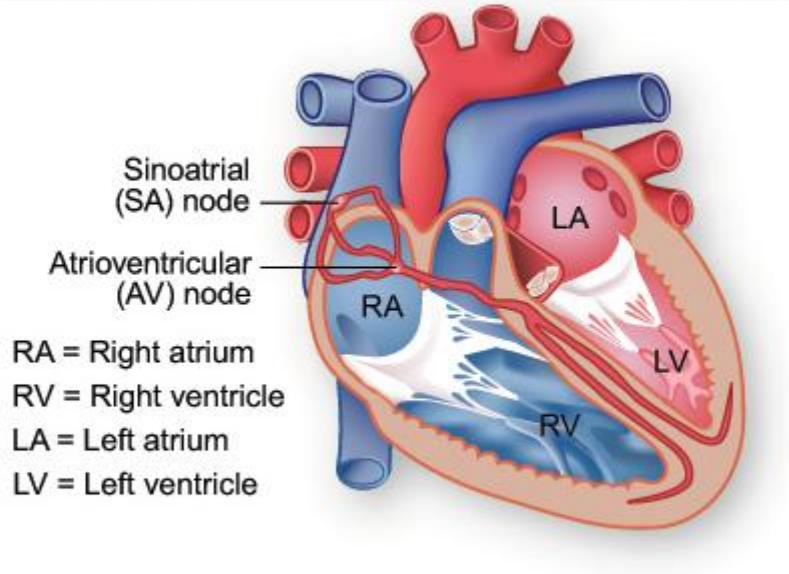
The pulmonary valve controls blood flow from the right ventricle into the pulmonary arteries, which carry blood to your lungs to pick up oxygen.

The mitral valve lets oxygen-rich blood from your lungs pass from the left atrium into the left ventricle.

The aortic valve opens the way for oxygen-rich blood to pass from the left ventricle into the aorta, your body's largest artery, where it is delivered to the rest of your body.



Heart Anatomy: Cardiac Conduction



Cardiac muscle cells contract spontaneously. These contractions are coordinated by the sinoatrial (SA) node which is also referred to as the pacemaker of the heart.

The SA node is located in the upper wall of the right atrium. When the SA node contracts it generates nerve impulses that travel throughout the heart wall causing both atria to contract.

When the impulses reach the atrioventricular (AV) node (lies on the right side of the septum that divides the atria) they are delayed for about a tenth of a second. This delay allows the atria to contract and empty their contents first.

The impulses are then sent down the atrioventricular bundle to the left and right ventricles.

At the base of the heart the atrioventricular bundles start to divide further into Purkinje fibers. When the impulses reach these fibers they trigger the muscle fibers in the ventricles to contract.

> **Note:** *The SA node sends electrical impulses at a certain rate, but the heart rate may still change depending on physical demands, stress, or hormonal factors.*

Cardiac Cycle (*heartbeat*)

The cardiac cycle is the sequence of events that occurs when the heart beats. There are two phases of the cardiac cycle.

In the diastole phase:

The right and left atria fills with blood.

As blood collects in the upper chambers, the heart's pacemaker sends out an electrical signal that causes the atria to contract. This contraction pushes blood through the tricuspid and mitral valves into the resting lower chambers (the right and left ventricles).

In the systole phase,

The second part of the pumping phase begins when the ventricles are full of blood. The electrical signals from the SA node travel along a pathway of cells to the ventricles, causing them to contract. This is called systole. As the tricuspid and mitral valves shut tight to prevent a back flow of blood, the pulmonary and aortic valves are pushed open. While blood is pushed from the right ventricle into the lungs to pick up oxygen, oxygen-rich blood flows from the left ventricle to the heart and other parts of the body.

After blood moves into the pulmonary artery and the aorta, the ventricles relax, and the pulmonary and aortic valves close. The lower pressure in the ventricles causes the tricuspid and mitral valves to open, and the cycle begins again.

> **Note:** *The heart normally beats about 60 to 80 times a minute when you are at rest*

The Circulatory System

The heart works as a pump that pushes blood to the organs, tissues, and cells of body. Blood delivers oxygen and nutrients to every cell and removes the carbon dioxide and waste products made by those cells.

Blood is carried from your heart to the rest of your body through a complex network of arteries, arterioles, and capillaries. Blood is returned to your heart through venules and veins.

Pulmonary circulation

The Pulmonary circulation is the portion of the cardiovascular system which transports oxygen-depleted blood away from the heart, to the lungs, and returns oxygenated blood back to the heart.

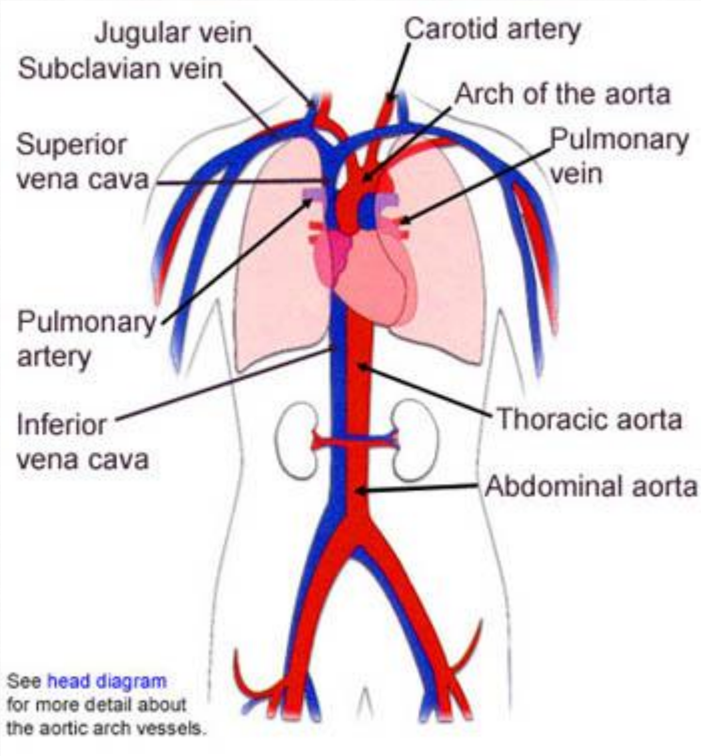
Oxygen deprived blood from the vena cava enters the right atrium of the heart and flows through the tricuspid valve into the right ventricle, from which it is pumped through the pulmonary semilunar valve into the pulmonary arteries which go to the lungs. Pulmonary veins return the now oxygen-rich blood to the heart, where it enters the left atrium before flowing through the mitral valve into the left ventricle. Then, oxygen-rich blood from the left ventricle is pumped out via the aorta, and on to the rest of the body.

Systemic circulation

Systemic circulation is the portion of the cardiovascular system which transports oxygenated blood away from the heart, to the rest of the body, and returns oxygen-depleted blood back to the heart.

In humans, blood is pumped from the strong left ventricle of the heart through arteries to peripheral tissues and returns to the right atrium of the heart through veins.

> **Note:** *Arterial blood carries oxygen, and venous blood carries carbon dioxide, However, one exception includes pulmonary arteries, which contain the most deoxygenated blood in the body, while the pulmonary veins contain oxygenated blood.*

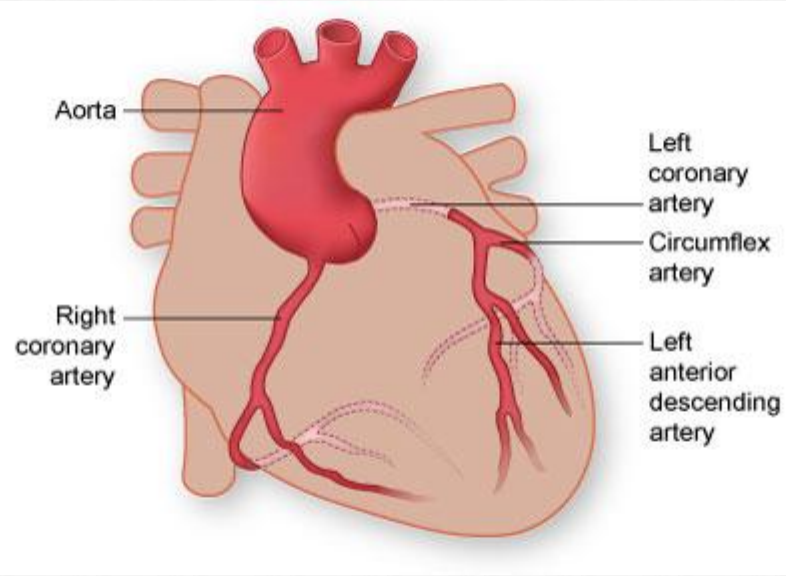


Coronary Circulation

The heart muscle, like every other organ or tissue in your body, needs oxygen-rich blood to survive. Blood is supplied to the heart by its own vascular system, called coronary circulation.

The aorta (the main blood supplier to the body) branches off into two main coronary blood vessels (also called arteries). These coronary arteries branch off into smaller arteries, which supply oxygen-rich blood to the entire heart muscle.

The right coronary artery supplies blood mainly to the right side of the heart. The left coronary artery, supplies blood to the left side of the heart.



Cardiac markers

Cardiac markers are biomarkers measured to evaluate heart function. Most of the early markers identified were enzymes, and as a result, the term "***cardiac enzymes***" is sometimes used. However, not all of the markers currently used are enzymes.

Cardiac markers or cardiac enzymes are proteins that leak out of injured myocardial cells through their damaged cell membranes into the bloodstream. Until the 1980s, the enzymes SGOT and LDH were used to assess cardiac injury. Now, the markers most widely used in detection of MI are MB subtype of the enzyme creatine kinase and cardiac troponins T and I as they are more specific for myocardial injury.

> **Note:** *An ECG still remains the most specific diagnostic tool in evaluating the patient with chest pain however, the initial ECG may be negative/non-diagnostic in > 40% of AMI cases*

> **Note:** *the perfect cardiac marker test, with a 100% early sensitivity + 100% specificity in diagnosing an AMI, does not exist different cardiac marker tests are used in varying combinations*

Why it is done?

Cardiac enzymes levels help diagnose chest pain or other signs and symptoms of a heart attack.

Limitations

Depending on the marker, it can take between 2 to 24 hours for the level to increase in the blood.

Additionally, determining the levels of cardiac markers in the laboratory takes time. Cardiac markers are therefore not useful in diagnosing a myocardial infarction in the acute phase. The clinical presentation and results from an ECG are more appropriate in the acute situation.

Lactate dehydrogenase (LDH)

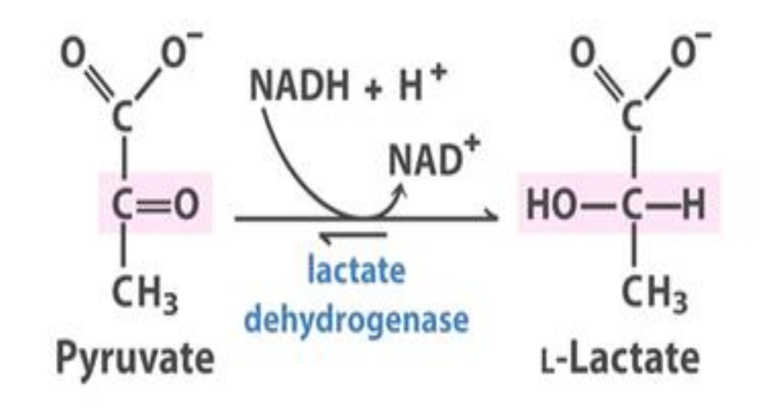
Overview:

Lactate dehydrogenase (LDH, or LD) is an enzyme that is found in almost all body tissues but only a small amount of it is usually detectable in the blood. It usually stays contained within the tissues cells. When cells are damaged or destroyed, however, they release LDH into the bloodstream, causing blood levels to rise. For this reason, LDH is used as a general marker of injury to cells.

Function

Lactate dehydrogenase catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD+.

It converts pyruvate, the final product of glycolysis to lactate when oxygen is absent or in short supply. This reaction is known as anaerobic homolactic fermentation and is an important way to regenerate NAD+ to allow glycolysis to continue .



LDH isoenzymes

LDH functions as a tetramer and is made of two kinds of subunits, H and M, each of which are encoded by a different gene. This results in 5 different isoenzymes (2 homotetramers and 3 heterotetramers).

The M subunit is found predominantly in anaerobic tissues including skeletal muscle and liver. The H subunit is more commonly found in tissues with a ready source of oxygen and that metabolize lactate including the heart and the brain.

- ***LDH-1 (4H)*** **- in the heart**
- ***LDH-2 (3H1M)*** **- in the reticuloendothelial system**
- ***LDH-3 (2H2M)*** **- in the lungs**
- ***LDH-4 (1H3M)*** **- in the kidneys**
- ***LDH-5 (4M)*** **- in the liver and striated muscle**

Usually LDH-2 is the predominant form in the serum. A LDH-1 level higher than the LDH-2 level (a "flipped pattern"), suggests myocardial infarction.

Sample:

Serum sample is used

Source of error

- Strenuous exercise can cause temporary elevations in LDH
- Hemolysis of blood can cause false positives

Interpretation:

Elevated levels of LDH and changes in the ratio of the LDH isoenzymes usually indicate some type of tissue damage. Usually LDH levels will rise as the cellular destruction begins, peak after some time period, and then begin to fall.

Cardiac disease:

It can be used as a marker of myocardial infarction. Following a myocardial infarction, levels of LDH will rise within ***24 to 48 hours*** ,peak at ***3-4 days*** and remain elevated for up to ***10 days***.

In this way, elevated levels of LDH can be useful for determining if a patient has had a myocardial infarction if they come to doctors several days after an episode of chest pain.

LDH level is directly proportional to the infraction size

Note: *LDH level can be elevated in other cardiac disease such as Myocarditis and rheumatic fever*

Non-cardiac disease:

Elevated levels of LDH may be seen in the following conditions:

- Hemolytic anemia and Pernicious anemia but it decrease with treatment.
- Liver disease: LDH increase in liver disease (mainly LD4 and LD5) but not as much as GOT and GPT . Toxic hepatitis and carcinoma of liver cause high elevation while Viral hepatitis and obstructive jaundice cause moderate elevation
- Muscular dystrophy: It increase in patients with progressive muscle dystrophy especially in early and middle stage of the disease then it decreases with progression of the disease
- Some cancers (especially lymphoma and leukemia) as cancer cells have a high rate of turnover with destroyed cells leading to an elevated LDH activity.

Total creatine kinase (CK)

Overview:

Creatine kinase (CK), also known as creatine phosphokinase (CPK) or phospho-creatine kinase, is an enzyme expressed by various tissues and cell types.

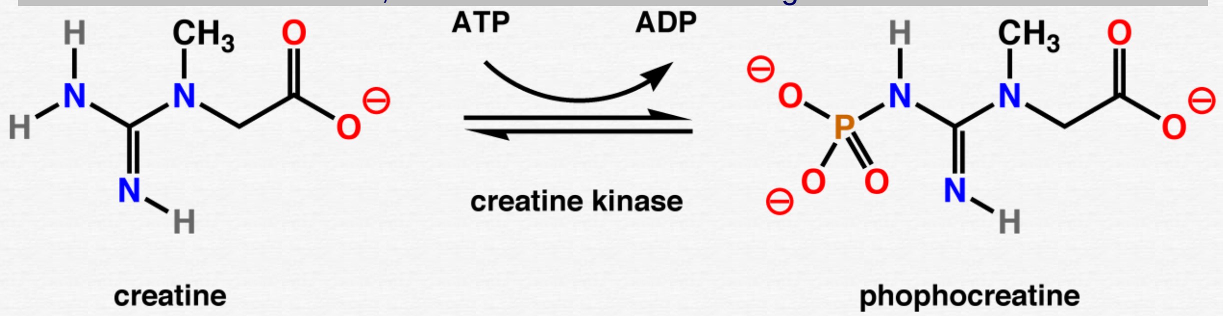
What is Creatine?

Creatine is produced by the body at rate of 1-2g/day from the amino acids, glycine, arginine and methionine. The liver is the major site of production but some is also produced in the kidney and pancreas. It exists as free creatine and creatine phosphate (CP).

Function

CK catalyses the conversion of creatine and consumes adenosine triphosphate (ATP) to create phosphocreatine and adenosine diphosphate (ADP).

This reaction is reversible, such that also ATP can be generated from PCr and ADP.



Role of phosphocreatine (PC)

In tissues and cells that consume ATP rapidly, especially skeletal muscle, phosphocreatine serves as an energy reservoir for the rapid buffering and regeneration of ATP in situ.

Thus when muscle contracts , ATP is consumed to form ADP , in this case CK catalyse the rephosphorylation of ADP to form ATP using Crp as the phosphorylation reservoir.

CK isoenzymes

In the cells, the CK enzymes consists of two subunits, which can be either B (brain type) or M (muscle type). There are, therefore, three different isoenzymes: CK-MM (CK3), CK-BB (CK1) and CK-MB (CK2).

- **CK-BB** occurs mainly in brain tissues, and its levels do rarely have any significance in bloodstream.
- Skeletal muscle expresses **CK-MM** (98%) and low levels of **CK-MB** (1%).
- The myocardium (heart muscle), in contrast, expresses **CK-MM** at 70% and **CK-MB** at 25–30%.

Sample:

Serum sample is used

Interpretation:

Cardiac disease:

CK total is elevated rapidly after MI

In the first ***4 to 6 hours*** after a heart attack, the concentration of CK in blood begins to rise. It reaches its highest level in ***18 to 24 hours*** and returns to normal within ***2 to 3 days***.

CK level is directly proportional to the infraction size

- **Muscular disease:**

Because most of the CPK in the body normally exists in muscle, a rise in the amount of CPK in the blood indicates that muscle damage has occurred.

Physiological rise

sever muscular exercise , cramps , repeated muscular injections cause transient CK rise for 2-4 days

Pathological rise

- Parasitic infection caused by trichinosis
- Convulsions and muscle spasms
- Duchenne's muscular dystrophy

  Caused by mutation in the gene of dystrophin protein. In this case high plasma activities are found from birth and before the onset of clinical signs

  During the early clinical stages at the disease , very high activity usually present but at terminal stage of the disease , the level tend to be fall when the mass of functioning muscle diminish with progression of the disease

Creatine kinase-MB (CK-MB)

serum CK-MB mainly comes from myocardial tissue so it is the first cardiac enzyme to be elevated after MI

In the first ***2 to 4 hours*** after a heart attack, the concentration of CK-MB in blood begins to rise. It reaches its highest level in ***12 to 24 hours*** and returns to normal within ***1 to 3 days***.

the sensitivity at 4 hours is < 50%, but the sensitivity should reach ~ 100% for AMI 10 – 12 hours after the onset of the chest pain

an AMI cannot be ruled-out before ~ 9 - 10 hours after the onset of symptoms; longer if the patient has ongoing chest pain

Why CK-MB is recommended?

- Used when very early conformation of diagnosis is required with in 4 h of the infarction
- When an increased CK total is suspected to be due to release of enzyme from skeletal muscle e.g. after intramuscular injections
- In patient suspected to having had a second infarction with few days and the first where the second peak rise of CK-MB is noted
- small females with a small total body muscle mass may have low serum CK levels, which may not rise above the threshold value after an AMI the more specific CK-MB test is recommended in these cases.

Angina pectoris

Angina pectoris, commonly known as angina, is severe chest pain due to ischemia (a lack of blood and hence oxygen supply) of the heart muscle, generally due to obstruction or spasm of the coronary arteries (the heart's blood vessels).

- **Causes:**

Coronary artery disease, the main cause of angina, is due to atherosclerosis of the cardiac arteries.

Myocardial ischemia comes about when the myocardia (the heart muscles) receive insufficient blood and oxygen to function normally because of decreased supply to the myocardia. This inadequate perfusion of blood and the resulting reduced delivery of oxygen and nutrients is directly correlated to blocked or narrowed blood vessels.

> **Note:** *There is a weak relationship between severity of pain and degree of oxygen deprivation in the heart muscle (i.e., there can be severe pain with little or no risk of a heart attack, and a heart attack can occur without pain).*

Myocardial infarction

Myocardial infarction (MI) or acute myocardial infarction (AMI), commonly known as a heart attack, is the interruption of blood supply to part of the heart, causing heart cells to die (necrosis). This is most commonly due to occlusion (blockage) of a coronary artery

- **Signs and symptoms**

- Classical symptoms of acute myocardial infarction include sudden chest pain (typically radiating to the left arm or left side of the neck)
- shortness of breath (dyspnea) occurs when the damage to the heart limits the output of the left ventricle, causing left ventricular failure and consequent pulmonary edema.
- nausea , vomiting and palpitations: These symptoms are likely induced by a massive surge of catecholamines from the sympathetic nervous system which occurs in response to pain
- excessive form of sweating (diaphoresis)
- anxiety: often described as a sense of impending doom.
- Loss of consciousness (due to inadequate cerebral perfusion and cardiogenic shock)

sudden death (frequently due to the development of ventricular fibrillation) can occur in myocardial infarctions.

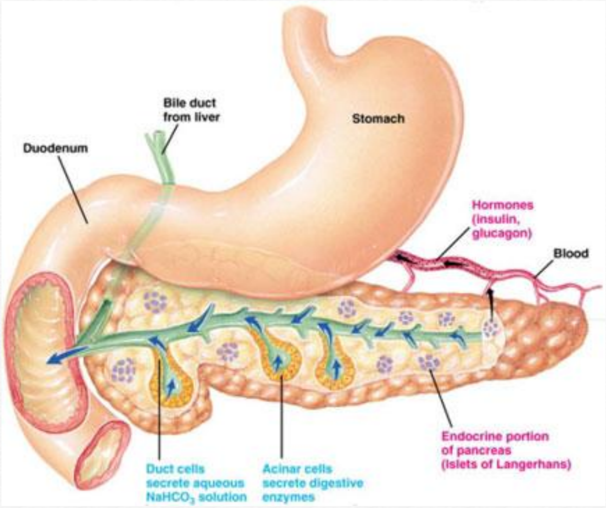
> **Note:** *Approximately one fourth of all myocardial infarctions are silent, without chest pain or other symptoms. These cases can be discovered later on electrocardiograms, or using blood enzyme tests. A silent course is more common in the elderly, in patients with diabetes mellitus and after heart transplantation, probably because the donor heart is not connected to nerves of the host.*

- **Risk factors**

Heart attacks are the leading cause of death for both men and women worldwide. Generally risk factors for atherosclerosis are generally risk factors for myocardial infarction:

Pancreatic function tests

The pancreas is a elongated organ, pinkish in color, that lies in close proximity to the duodenum. It is covered with a very thin connective tissue capsule which extends inward as septa, partitioning the gland into lobules.



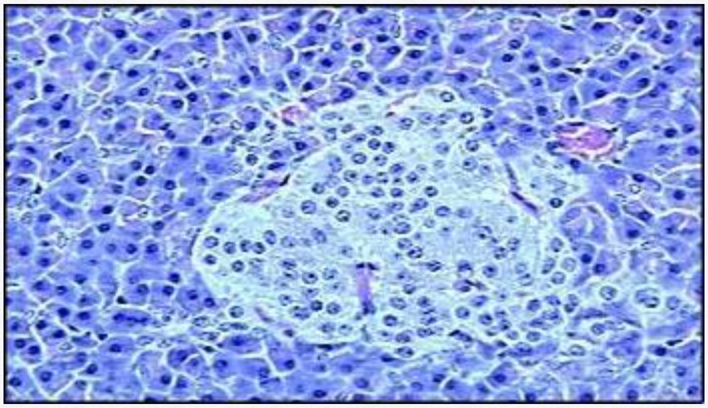
Pancreas anatomy

Pancreas consists of:

- Head: Lies within the concavity of the duodenum
- Neck: The constricted part between the head and the body
- Body: It lies behind stomach
- Tail: It is the left end of the pancreas. It lies in contact with the spleen and runs in the lienorenal ligament

Pancreas histology

The bulk of the pancreas is composed of pancreatic exocrine cells and their associated ducts. Embedded within this exocrine tissue are roughly one million small clusters of cells called the Islets of Langerhans, which are the endocrine cells of the pancreas.



Exocrine Tissue

The Acinus

The exocrine pancreas is classified as a compound tubuloacinous gland. The cells that synthesize and secrete digestive enzymes are arranged in grape-like clusters called acini,

Pancreatic Ducts

Digestive enzymes from acinar cells ultimately are delivered into the duodenum. Secretions from acini flow out of the pancreas through a tree-like series of ducts.

Pancreatic ducts are classified into four types which are discussed here beginning with the terminal branches which extend into acini.

- Intercalated ducts receive secretions from acini.
- Intralobular ducts They receive secretions from intercalated ducts.
- Interlobular ducts are found between lobules, they transmit secretions from intralobular ducts to the major pancreatic duct.
- The main pancreatic duct received secretion from interlobular ducts and penetrates through the wall of the duodenum.

Endocrine Tissue

If you examine a section of pancreas at low magnification, you will undoubtedly observe distinctive areas of pale staining embedded within the exocrine tissue of lobules. These are the Islets of Langerhans,

Islets contain several different endocrine cell types. The most abundant are beta cells, which produce insulin, and alpha cells, which secrete glucagon.
The endocrine cells within islets are arranged as irregular cords around abundant capillaries, which receive the secreted hormones for delivery into the systemic circulation.

Pancreas function

The pancreas is a dual-function gland, having features of both endocrine and exocrine glands.

The endocrine function

The part of the pancreas with endocrine function is made up of approximately a million cell clusters called islets of Langerhans. Four main cell types exist in the islets. They are relatively difficult to distinguish using standard staining techniques, but they can be classified by their secretion: α cells secrete glucagon (increase Glucose in blood), β cells secrete insulin (decrease Glucose in blood), δ cells secrete somatostatin (regulates/stops α and β cells), and PP cells secrete pancreatic polypeptide

Control of pancreatic endocrine secretion

The pancreas receives regulatory innervation via hormones in the blood and through the autonomic nervous system. These two inputs regulate the secretory activity of the pancreas.

Sympathetic (adrenergic)	Parasympathetic (muscarinic)
α2: decreases secretion from beta cells, increases secretion from alpha cells	M3 increases stimulation of alpha cells and beta cells

The exocrine function

secretions are made up of 2 components, the alkaline pancreatic fluid & the digestive enzymes.

The digestive enzymes

The pancreas secretes a three major groups of enzymes are critical to efficient digestion:

1. Proteases

Digestion of proteins is initiated by pepsin in the stomach, but the bulk of protein digestion is due to the pancreatic proteases. Several proteases are synthesized in the pancreas and secreted into the lumen of the small intestine. The two major pancreatic proteases are trypsin and chymotrypsin, which are synthesized and packaged into secretory vesicles as an the inactive proenzymes trypsinogen and chymotrypsinogen.

2. Pancreatic Lipase

A major component of dietary fat is triglyceride, or neutral lipid. A triglyceride molecule cannot be directly absorbed across the intestinal mucosa. Rather, it must first be digested into a 2-monoglyceride and two free fatty acids. The enzyme that performs this hydrolysis is pancreatic lipase, which is delivered into the lumen of the gut as a constituent of pancreatic juice.

3. Pancreatic Amylase

The major dietary carbohydrate for many species is starch, a storage form of glucose in plants. Amylase (technically alpha-amylase) is the enzyme that hydrolyses starch to maltose (a glucose-glucose disaccharide),

The alkaline fluid

Epithelial cells in pancreatic ducts are the source of the bicarbonate and water secreted by the pancreas. Bicarbonate is a base and critical to neutralizing the acid coming into the small intestine from the stomach.
The alkaline fluid is primarily responsible for neutralizing gastric acid secretions, thus providing an optimal environment for duodenal digestive enzyme activity.

Control of pancreatic exocrine secretion

Like the stomach, the pancreas is innervated by the vagus nerve, which applies a low level stimulus to secretion in response to anticipation of a meal. However, the most important stimuli for pancreatic secretion comes from three hormones secreted by the enteric endocrine system:

Cholecystokinin: Its secretion is strongly stimulated by the presence of partially digested proteins and fats in the small intestine. cholecystokinin is released into blood and binds to receptors on pancreatic acinar cells, ordering them to secrete large quantities of digestive enzymes.

Secretin: Secretin is secreted in response to acid in the duodenum, which of course occurs when acid-laden chyme from the stomach flows through the pylorus. The predominant effect of secretin on the pancreas is to stimulate duct cells to secrete water and bicarbonate.

Gastrin: This hormone, which is very similar to cholecystokinin, is secreted in large amounts by the stomach in response to gastric distention and irritation. In addition to stimulating acid secretion by the parietal cell, gastrin stimulates pancreatic acinar cells to secrete digestive enzymes.

Pancreatic function test

A number of tests are used to diagnose pancreas problems, including the pancreatic enzymes amylase and lipase.

Why pancreatic enzymes are assayed?

- Pancreatic enzymes may be ordered if you show symptoms of a pancreatic disorder, such as severe abdominal pain, fever, loss of appetite, or nausea.
- To diagnose and monitor acute or chronic pancreatitis and other disorders that may involve the pancreas.
- They are sometimes used to monitor treatment of cancers involving the pancreas and after the removal of gallstones that have caused gallbladder attacks.

Amylase test

Overview

The major dietary carbohydrate for many species is starch, a storage form of glucose in plants. Amylase (technically alpha-amylase) is the enzyme that hydrolyses starch to maltose (a glucose-glucose disaccharide), as well as the trisaccharide maltotriose and small branch points fragments called limit dextrins. The major source of amylase in all species is pancreatic secretions, although amylase is also present in saliva of humans.

Amylase is a digestive enzyme that normally acts extracellularly to cleave starch by hydrolysis of internal alpha-1,4-glycoside bonds, which results in the production of maltose and oligosaccharides.

The α-amylases are calcium metalloenzymes, completely unable to function in the absence of calcium.

Isoamylases

The amylase present in blood and urine of normal individuals is predominantly of pancreatic and salivary origin.

Amylase of pancreatic and salivary origin is abbreviated to P-type and S-type amylase

Salivary amylase (ptyalin) S-isoenzyme

This form of amylase is also called ptyalin. It will break large, insoluble starch molecules into soluble starches (amylodextrin, erythrodextrin, achrodextrin) producing successively smaller

starches and ultimately maltose. Ptyalin acts on linear α(1,4) glycosidic linkages. Salivary amylase is inactivated in the stomach by gastric acid.

Pancreatic amylase (diastase) P-isoenzyme

Pancreatic α-amylase randomly cleaves the α(1-4) glycosidic linkages of amylose to yield dextrin, maltose or maltotriose. It adopts a double displacement mechanism with retention of anomeric configuration.

Sample

Serum sample is used

> **Note:** *Plasma specimens should be avoided for amylase determination because amylase is a calcium-containing enzyme, and falsely low enzyme activities are obtained from such specimens.*
>
> **Note:** *In serum it is stable for 1 week at room temperature and for at least 6 months under refrigeration in well-sealed containers.*
>
> **Note:** *In pancreatitis with associated hypertriglyceridemia, serum amylase levels may be erroneously normal. This is attributed to an inhibitor associated with triglyceride elevations that interferes with the assay for the enzyme.*

Normal level

Adults: 60 to 180 U/L

> **Note:** *In pediatrics amylase level remains low for the first two months and increases to adult values by the end of the first year of life.*

Results interpretation

Acute or chronic pancreatitis is associated with increases in the P-type isoamylase.

Acute pancreatitis

Acute pancreatitis or acute pancreatic necrosis is a sudden inflammation of the pancreas.

Symptoms and signs

The most common symptoms and signs include:

- Severe epigastric pain radiating to the back
- Nausea
- vomiting
- diarrhea
- loss of appetite
- Fever/chills

Causes

- **Gallstones:** gallstone that travel down the common bile duct and which subsequently get stuck in the Ampulla of Vater can cause obstruction in the outflow of pancreatic juices from the pancreas into the duodenum. The backflow of these digestive juices causes lysis (dissolving) of pancreatic cells and subsequent pancreatitis.
- Virus infection such as mumps, Epstein-Barr virus, and Cytomegalovirus.
- Autoimmune disease such as systemic lupus erythematosus)
- Carcinoma of the head of pancreas
- Ascaris blocking pancreatic outflow

Pathogenesis

In acute pancreatitis, the worst offender among these enzymes may well be the protease trypsinogen which converts to the active trypsin. Trypsin is most responsible for auto-digestion of the pancreas which in turn causes the pain and complications of pancreatitis.

Diagnosis

In acute pancreatitis, amylase in the blood often start to increase within 2-3 h of injury to the pancreas, peaks 2-30 h (increases to 4 to 6 times higher than the highest reference value) then return to normal 2-6 days.

Approximately 20% of patients with pancreatitis have normal or near-normal amylase activity.

Why amylase level increase in pancreatic disease?

Amylase is usually present in the blood in small quantities. When cells in the pancreas are injured, as in pancreatitis, increased amounts of amylase find their way into the bloodstream.

Other clinical finding

- Serum CRP are elevated and peaks 3 days after onset of pain.
- Blood glucose >250 mg/dl
- Glycosuria appears in 25% of patients.
- WBC is slightly to moderately increased (10,000–20,000/μL).

Chronic pancreatitis

Chronic pancreatitis is a long-standing inflammation of the pancreas that alters its normal structure and functions. It can present as episodes of acute inflammation, or as chronic damage with persistent pain or malabsorption.

Symptoms

Patients with chronic pancreatitis usually present with:

- persistent abdominal pain or steatorrhea resulting from malabsorption of the fats in food.
- Diabetes is a common complication due to the chronic pancreatic damage and may require treatment with insulin.

Causes

At least 70% of adult cases are caused by chronic alcohol use, and most patients have consumed more than 150 g/day of alcohol.

some cases of chronic pancreatitis are of undetermined or idiopathic origin.

Chronic pancreatitis are major risk factors for developing pancreatic cancer.

Diagnosis

In chronic pancreatitis, amylase levels initially will be moderately elevated but often decrease over time with progressive pancreas damage.

Note: *Decreased blood amylase levels in a person with pancreatitis symptoms may indicate permanent damage to the amylase-producing cells in the pancreas.*

Pancreatic carcinoma

Serum amylase may be elevated in patients with pancreatic carcinoma, but too late to be diagnostically useful.

An increase of serum amylase >7 to 10 days after acute pancreatitis may suggests an associated cancer of pancreas.

limitation

The major limitation of using serum amylase measurement in diagnosing pancreatitis is the lack of specificity because numerous clinical situations can result in elevated amylase such as:

- Parotitis which is associated with increases in the S-type isoamylase.
- Gut diseases, including intestinal obstruction, appendicitis, and peritonitis, usually result in increased P-type isoamylase because of increased absorption of amylase from the intestinal lumen.
- Ectopic amylase production by lung, ovary, breast and colon malignancies

What Affects the Test

- Some drugs that may cause amylase to rise include aspirin, diuretics, oral contraceptives, corticosteroids, and opiates.
- Drinking a large amount of alcohol before the test.

Lipase test

Overview

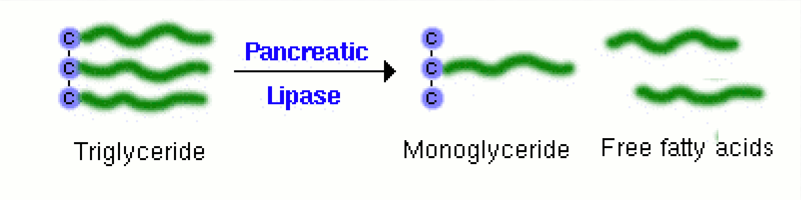
A lipase is a water-soluble enzyme that catalyzes the hydrolysis of ester bonds in water–insoluble, lipid substrates.
Lipases perform essential roles in the digestion, transport and processing of dietary lipids such as triglycerides.

Human lipases

The main lipases of the human digestive system are human pancreatic lipase (HPL) which are secreted by the pancreas. Humans also have several other related enzymes, including hepatic lipase (HL), endothelial lipase, and lipoprotein lipase. Not all of these lipases function in the gut

Function

A major component of dietary fat is triglyceride, or neutral lipid. A triglyceride molecule cannot be directly absorbed across the intestinal mucosa. Rather, it must first be digested into a 2-monoglyceride and two free fatty acids. The enzyme that performs this hydrolysis is pancreatic lipase, which is delivered into the lumen of the gut as a constituent of pancreatic juice.
Sufficient quantities of bile salts must also be present in the lumen of the intestine in order for lipase to efficiently digest dietary triglyceride and for the resulting fatty acids and monoglyceride to be absorbed.



Note: *Pancreatic lipase has recently been in the limelight as a target for management of obesity. The drug orlistat (Xenical) is a pancreatic lipase inhibitor that interferes with digestion of triglyceride and thereby reduces absorption of dietary fat.*

Sample

Serum is the specimen of choice for blood lipase assays.

Note: *Serum lipase is stable up to 1 week at room temperature and may be kept stable longer if it is refrigerated or frozen.*

Interfering Factors

- EDTA anticoagulant interferes with test.
- Lipase is increased in about 50% of patients with chronic renal failure.
- Lipase increases in patients undergoing hemodialysis.

Normal level

Adults: 0-160 U/L

Results interpretation

Acute pancreatitis

In acute pancreatitis, Lipase in the blood often start to increase within 4-8 h of injury to the pancreas, peaks 24 h (increases to 5 to 10 times higher than the highest reference value) then return to normal 8-14 days.

> **Note:** *In acute pancreatitis, elevated amylase levels usually parallel lipase concentrations, although lipase levels may take a bit longer to rise than blood amylase levels and will remain elevated longer.*

> **Note:** *Increased lipase activity rarely lasts longer than 14 days, and prolonged increases suggest a poor prognosis*

Chronic pancreatitis

Lipase eleviated but normal or marginal activity is seen because destruction of pancreatic cells in the late stage results in the reduction of the amount of enzyme that enter the serum

limitation

Although determination of serum lipase has diagnostic advantages over serum amylase for acute pancreatitis, it is not specific for acute pancreatitis. Serum lipase may also be elevated in nonpancreatic conditions including renal diseases, various abdominal diseases such as cholecystitis, intestinal obstruction, duodenal ulcer, and liver disease, as well as alcoholism, and diabetic ketoacidosis.

Diabetes profile

Glucose homeostasis

Glucose is the primary source of energy for body's cell, lipids (in the form of fats and oils) being primarily a compact energy store. It is transported from the intestines or liver to body cells via the bloodstream, and is absorbed by body cells with the intervention of the hormone insulin normally produced by the body.

Blood sugar regulation

A body's homeostatic mechanism keeps blood glucose levels within a narrow range. It is composed of several interacting systems, of which hormone regulation is the most important.

There are two types of mutually antagonistic metabolic hormones affecting blood glucose levels:

- **catabolic hormones** (such as glucagon, growth hormone, cortisol and catecholamines) which increase blood glucose;
- **anabolic hormone** (insulin), which decreases blood glucose.

Mechanism of insulin action

When levels of blood sugar rise, whether as a result of glycogen conversion, or from digestion of a meal, a hormone is released from beta cells found in the Islets of Langerhans in the pancreas. This hormone, insulin, causes the liver to convert more glucose into glycogen (this process is called glycogenesis), and to force about 2/3 of body cells (primarily muscle and fat tissue cells) to take up glucose from the blood through the GLUT4 transporter, thus decreasing blood sugar. When insulin binds to the receptors on the cell surface, vesicles containing the GLUT4 transporters come to the plasma member and fuse together by the process of exocytosis and thus enabling a facilitated diffusion of glucose into the cell. As soon as the glucose enters the cell, it is phosphorylated into Glucose-6-Phosphate in order to preserve the concentration gradient so glucose will continue to enter the cell.

Hyperglycemia

Hyperglycaemia or high blood sugar is a condition in which an excessive amount of glucose circulates in the blood plasma.

This is generally a blood glucose level of 180 mg/dl, but symptoms and effects may not start to become noticeable until later numbers like 270-360 mg/dl.

Causes of hyperglycemia:

- **Persistent hyperglycemia** This is can be seen in:
  - Diabetes mellitus
  - Hyperadrenocorticism (Cushing's Syndrome)
  - Acromegaly
  - Hyperthyroidism
- **Transient hyperglycemia** This is can be seen in:
  - Alimentary glucosuria

    Glycosuria developing after the ingestion of a moderate amount of sugar or starch, (usually following intake of low carbohydrate diet for about week) which normally is disposed of without appearing in the urine, because rate of intestinal absorption exceeds capacity of the liver and the other tissues to remove the glucose, thus allowing blood glucose levels to become high enough for renal excretion to occur.

- Hyperglycemia occurs naturally during times of infection and inflammation
- Stress-related hyperglycemia due to epinephrine secretion.
- Phaechromocytoma due to epinephrine secretion.
- pancreatitis, may induce a mild, transient hyperglycemia
- Severe liver disease may cause transient hyperglycemia (following meal)

Symptoms of hyperglycemia:

The following symptoms may be associated with acute or chronic hyperglycemia, with the first three comprising the classic hyperglycaemic triad:

- Polyphagia - frequent hunger, especially pronounced hunger
- Polydipsia - frequent thirst, especially excessive thirst
- Polyuria - frequent urination, especially excessive urination
- Blurred vision
- Weight loss
- Poor wound healing (cuts, scrapes, etc.)
- Dry mouth
- Dry or itchy skin
- Tingling in feet or heels
- Recurrent infections such as vaginal yeast infections, or groin rash.

Hypoglycemia

Hypoglycemia is currently defined as a blood glucose value of less than 50 mg/dl. Clinically, hypoglycemia is defined by Whipple's triad: low plasma glucose level, symptoms consistent with hypoglycemia, and resolution of symptoms with correction of the low glucose level.

Causes of hypoglycemia:

- **Fasting hypoglycemia**
  - Pancreatic disorders
    - Islet beta-cell hyperfunction (adenoma, , hyperplasia)
    - Islet alpha-cell hypofunction or deficiency
  - Pituitary-adrenal disorders
    - Hypopituitarism
    - Addison's disease

- **Postprandial (reactive) hypoglycemia**
  - Alimentary hypoglycemia (previous gastrointestinal [GI] surgery), peptic ulcer disease, disordered GI motility syndromes.
  - Hormonal (hypothyroidism; deficiency of cortisol, epinephrine, glucagon, or growth hormone)
  - Deficiency of early hepatic gluconeogenesis (fructose-1,6-diphosphatase deficiency)
  - Drugs (alcohol, lithium)

- **Neonatal hypoglycemia**

Hypoglycemia is a common problem in critically ill or extremely low birth weight infants.
neonatal hypoglycemia may be caused by:

- Prematurity
- Maternal hyperglycemia due to diabetes or iatrogenic glucose administration
- Prolonged fasting (e.g., due to inadequate breast milk or condition interfering with feeding)
- Inborn errors of carbohydrate metabolism such as glycogen storage disease

Symptoms of hypoglycemia:

The symptoms of hypoglycemia can be divided into two categories as follows. Adrenergic symptoms are catecholamine-mediated and Neuroglycopenic symptoms.

- **Adrenergic manifestations**
  - Nervousness
  - Palpitations, tachycardia
  - Sweating, feeling of warmth
  - Pallor, coldness
  - Dilated pupils
  - headaches
  - weakness

- **Neuroglycopenic manifestations**
  - Abnormal mentation, impaired judgment
  - Staring, "glassy" look, blurred vision, double vision
  - Difficulty speaking
  - Confusion
  - Convulsions, and coma.

Pre-Diabetes

Pre-diabetes (is also called impaired glucose tolerance (IGT) or impaired fasting glucose (IFG)) is a condition where people have blood glucose levels that are higher than normal but not yet high enough to be diagnosed as diabetes. Most people who develop type 2 diabetes have pre-diabetes first.

Most people who have pre-diabetes are unaware that they have it or that they are at risk of developing diabetes and cardiovascular disease.

People with pre-diabetes often develop type 2 diabetes within 10 years.

Diagnostic Criteria for Impaired glucose tolerance

- FPG 100–125 mg/dl
- OGTT 140–199 mg/dl

Diabetes Mellitus

Diabetes mellitus is impaired insulin secretion and variable degrees of peripheral insulin resistance leading to hyperglycemia. Early symptoms are related to hyperglycemia and include polydipsia, polyphagia, and polyuria. Later complications include vascular disease, peripheral neuropathy, and predisposition to infection. Diagnosis is by measuring plasma glucose.

Symptoms and Signs

The most common symptoms of DM are those of hyperglycemia: an osmotic diuresis caused by glycosuria leading to urinary frequency, polyuria, and polydipsia that may progress to orthostatic hypotension and dehydration. Severe dehydration causes weakness, fatigue, and mental status changes. Symptoms may come and go as plasma glucose levels fluctuate. Hyperglycemia can also cause weight loss, nausea and vomiting, and blurred vision, and it may predispose to bacterial or fungal infections.

❖ Types

There are 2 main categories of diabetes mellitus (DM)—type 1 and type 2, which can be distinguished by a combination of features.

General Characteristics of Types 1 and 2 Diabetes Mellitus

Characteristic	Type 1 (IDDM)	Type 2 (NIDDM)
Age at onset	Most commonly < 30 yr	Most commonly > 30 yr
Associated obesity	No	Very common
ketoacidosis	Yes	No
Plasma levels of endogenous insulin	Extremely low to undetectable	Variable; may be low, normal, or elevated depending on degree of insulin resistance and insulin secretary defect
Genetic predisposition	Moderate ≤ 50%	strong > 90%
responds to oral antihyperglycemic drugs	No	Yes, initially in many patients
Treatment	The pancreas produces very little or no insulin at all in people with type 1 diabetes. **All patients with type 1 diabetes will eventually require insulin.** **Dosing** — When insulin treatment is started, small doses are generally recommended; the dose is adjusted over days, weeks, and months, once the body's response to insulin is known. During this time, you will monitor blood sugar level several times per day to determine the optimal dose of insulin.	Treatment goals for type 2 diabetic patients are related to effective control of blood glucose, blood pressure and lipids to minimize the risk of long-term consequences associated with diabetes. **The targets are:** - Preprandial blood glucose: (72 to 108 mg/dl) - 2-hour postprandial blood glucose: (90 to 144 mg/dl) **Sulfonylureas** Sulfonylureas (Amaryl) were the first widely used oral hypoglycemic medications. They, triggering insulin release by direct action on the pancreatic beta cells.

❖ Metabolic complications of D. M.

► *Diabetic ketoacidosis:*

DKA is a state of uncontrolled catabolism triggered by a relative or absolute deficiency in circulating insulin. The triad of DKA is metabolic acidosis (pH < 7.35), hyperglycemia (blood glucose level usually >250 mg/dL), and positive ketones in the urine or blood. The relative or absolute deficiency of insulin is accompanied by a reciprocal elevation in counter-regulatory hormones (glucagon, epinephrine, growth hormone, and cortisol), causing increased glucose production by the liver (gluconeogenesis) and catabolism of fat (lipolysis). Lipolysis provides the

substrate (free fatty acids) for the uncontrolled production of ketones by the liver. The production of ketones leads to acidosis.

> **Note:** *DKA is most often associated with type 1 (insulin-dependent) diabetes. However, it also may occur in the older patients with type 2 (non-insulin-dependent) diabetes, particularly when associated with a major intercurrent illness.*

- ***Hyperglycemic coma:***

Ketotic hyperglycemic coma: hyperglycemia, glucosuria and urinary loss of water and Na, ketonemia, ketonuria, metabolic acidosis and hyperkalemia. Mostly occurs in IDDM.

Non-ketotic hyperglycemic coma: It is due to poor renal function in elderly patients with NIDDM and greater loss of water and electrolytes. The residual insulin can correct fat but not glucose homeostasis.

Systemic complications of D. M.

Diabetic retinopathy

Refers to progressive pathologic alterations in the retinal microvasculature, leading to areas of retinal nonperfusion, increased vascular permeability, and the pathologic proliferation of retinal vessels. Blindness occurs 25 times more frequently in diabetic patients than in control subjects and is seen most often after the disease has been present for at least 15 years.

Diabetic nephropathy

Diabetic nephropathy is clinically defined by persistent proteinuria greater than 500 mg/24 hours in a person with diabetes without other renal disease. It is the leading cause of end-stage renal disease requiring dialysis or transplantation.
Both type 1 and type 2 diabetes cause renal disease. Compared to type 1, a slightly smaller and imperfectly defined proportion of type 2 patients progress to ESRD, but they represent more than 90% of those receiving renal replacement therapy with the diagnosis of diabetes.

Diabetic neuropathy

is a common and troublesome complication of diabetes mellitus, leading to great morbidity and mortality and resulting in a huge economic burden for care of the patient with diabetes mellitus.
Diabetic neuropathy is a heterogeneous disorder that encompasses a wide range of abnormalities affecting proximal and distal peripheral sensory and motor nerves as well as the autonomic nervous system. The major morbidity associated with somatic neuropathy is foot ulceration, the precursor of gangrene and limb loss.

Diabetic cardiomyopathy

It is a disorder of the heart muscle in people with diabetes. It can lead to inability of the heart to circulate blood through the body effectively, a state known as ***heart failure***, with accumulation of fluid in the lungs (***pulmonary edema***) or legs (***peripheral edema***). Most heart failure in people with diabetes results from coronary artery disease.
four main causes are responsible for the development of heart failure in DCM:

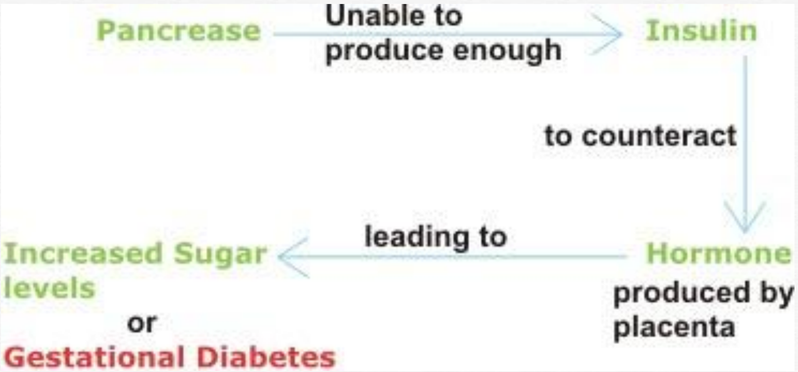
- Microangiopathy (characterized as subendothelial and endothelial fibrosis in the coronary microvasculature of the heart.) and related endothelial dysfunction
- Autonomic neuropathy
- Metabolic alterations that include abnormal glucose use and increased fatty acid oxidation, generation and accumulation of free radicals
- Alterations in ion homeostasis, especially calcium transients.

Gestational diabetes

Gestational diabetes is diabetes that is found for the first time when a woman is pregnant.

❖ **Causes of gestational diabetes**

In gestational diabetes, the pancreas is not at fault. Instead, the problem is in the placenta. During pregnancy, the placenta provides the baby with nourishment. It also produces a number of hormones that interfere with the body's usual response to insulin. This condition is referred to as "insulin resistance." Most pregnant women do not suffer from gestational diabetes, because the pancreas works to produce extra quantities of insulin in order to compensate for insulin resistance. However, when a woman's pancreas cannot produce enough extra insulin, blood levels of glucose stay abnormally high, and the woman is considered to have gestational diabetes.



❖ **Risk factors of gestational diabetes**

- I have a parent, brother, or sister with diabetes.
- I am 25 years old or older.
- I am overweight.
- I have had gestational diabetes before, or I have given birth to at least one baby weighing more than 9 pounds.

❖ **How will gestational diabetes affect the mother?**

Often, women with gestational diabetes have no symptoms. However:

- gestational diabetes may increase your risk of high blood pressure during pregnancy.
- increase your risk of a large baby and the need for cesarean section at delivery

Note: *gestational diabetes will probably go away after your baby is born. However, you will be more likely to get type 2 diabetes later in your life. You may also get gestational diabetes again if you get pregnant again.*

❖ **How will gestational diabetes affect the baby?**

Untreated or uncontrolled gestational diabetes can mean problems for your baby, such as:

- being born very large and with extra fat; this can make delivery difficult and more dangerous for your baby
- low blood glucose right after birth
- breathing problems

Glucosuria:

It occurs when plasma glucose concentration exceeds its renal tubular absorptive capacity [renal glucose threshold: (l80 mg/d)].

- ❖ Hyperglycemia without glucosuria occurs when the renal threshold for glucose is raised.
- ❖ There may be glucosuria without hyperglycemia as in low renal threshold for glucose (renal glucosuria).

Renal glucosuria

Also known as benign glycosuria, or Familial renal glycosuria.
It is a rare condition in which the glucose is excreted in the urine despite normal or low blood glucose levels (glucose in the urine without hyperglycemia).

Cause:

Renal glycosuria is due to improper functioning of the proximal renal tubules and may due to:

- Inflammatory renal disease (e.g., acute GN, nephrosis)
- Fanconi syndrome
  is a disorder in which the proximal tubular function of the kidney is impaired, resulting in decreased re-absorption of electrolytes and nutrients back into the bloodstream as glucose, amino acids, uric acid, phosphate and bicarbonate.
- Isolated renal glycosuria
  When renal glycosuria occurs as an isolated finding with normal kidney function, the condition is thought to be inherited as an autosomal trait (It is associated with gene, coding the sodium glucose cotransporter). And In most affected individuals, the condition causes no apparent symptoms or serious effects.

Blood glucose test

Overview:

Blood sugar concentration, or glucose level, refers to the amount of glucose present in the blood of a human. Normally, in mammals the blood glucose level is maintained at a reference range between about 70 and 120 mg/dl
Failure to maintain blood glucose in the normal range leads to conditions of persistently high (hyperglycemia) or low (hypoglycemia) blood sugar.

Why Get Tested?

- To determine if your blood glucose level is within healthy ranges
- To screen for, diagnose, and monitor hyperglycemia, hypoglycemia, diabetes, and pre-diabetes
- To screen for gestational diabetes during pregnancy

Sample:

Serum or plasma

Patient must be fasting for 6-8 hours before doing FBS

Serum or plasma must be separated as soon as possible from RBCs

Blood samples in which serum is not separated from blood cells will show glucose values decreasing at rate of 3% to 5% per hour at room temperature.

types:

Several different types of blood glucose tests are used.

- **Fasting blood sugar (FBS)** measures blood glucose after you have not eaten for at least 8 hours. It often is the first test done to check for diabetes.
- **2-hour postprandial blood sugar** measures blood glucose exactly 2 hours after you eat a meal.
- **Random blood sugar (RBS)** measures blood glucose regardless of when you last ate. Several random measurements may be taken throughout the day. Random testing is useful because glucose levels in healthy people do not vary widely throughout the day. Blood glucose levels that vary widely may indicate a problem. This test is also called a casual blood glucose test.

Procedure:

	Blank	Standard	Test
Reagent	1 ml	1 ml	1 ml
Standard	………..	10 ul	………..
Sample	………..	………..	10 ul
Incubate at 37 degree for 10 min then read absorbance against blank at wave length **520 nm**			
Calculation: sample concentration = (Absorbance of test / Absorbance of standard) X 100			

Normal:

Fasting glucose Level	Indication
From 70 to 99 mg/dl	Normal fasting glucose
From 100 to 125 mg/dl	(pre-diabetes)
126 mg/dl and above on more than one testing occasion	Diabetes

Interpretation:

- According to the WHO criteria, a fasting venous plasma glucose of 126 mg/dl or more in two occasions is diagnostic of D.M.
- Blood glucose 2 hours after meal is considered the simplest screening test for D.M. Normally it should be 120mg/dl. Post prandial levels above 200mg/dl are diagnostic.
- Alternatively a random venous plasma glucose of 200mg/dl or more establishes the diagnosis.

The oral glucose tolerance test is regarded as a definitive test for confirming a provisional diagnosis of D. M. in borderline cases. (Pre-Diabetes cases 110 - 125 mg/dl)

Oral glucose tolerance curve

Overview

A glucose tolerance test is a medical test in which glucose is given and blood samples taken afterward to determine how quickly it is cleared from the blood. The test is usually used to test for diabetes, insulin resistance, and sometimes reactive hypoglycemia or rarer disorders of carbohydrate metabolism. In the most commonly performed version of the test, an oral glucose tolerance test (OGTT), a standard dose.

Why Get Tested?

- This test is used to confirm or rule out diabetes in cases where there is absence of definitive diagnosis of DM.
- The diagnosis of Impaired glucose tolerance especially in pregnancy (gestational diabetes).
- It is a definitive test for diagnosing hypoglycemia.
- To diagnose patient with persistent hyperglycemia with out glucosuria (blood glucose below the renal threshold)
- May be used to test small intestine absorption function.

Principle of test

When glucose is given by oral route alteration of blood glucose level concentration takes three phases

Phase I

Occurred during absorption period. The rate of glucose absorption in circulation exceeds that of removal so blood glucose level is high.

Phase II

Blood glucose rises and reach maximum level in 1 hour and Insulin secretion is stimulated so the peak level of glucose is reached and then begin to fall (rate of removal exceeds that of entry).

Phase III

This part of curve represent glucose utilization by the tissue in response to insulin secretion (ascending limb) so the glucose level reach to the original value after 2 hours

Note: *any cause that leads to impairing glucose absorption as diarrhea make the glucose level reaches the maximum in much slower rate (more than 1 hour)*

Note: *in DM glucose return to fasting level in much slower rate*

Procedure

- The patient should be on balanced diet containing normal daily requirements of carbohydrates at least for 2-3 days before the test
- The patient is instructed to fast (water is allowed) for 8-12 hours prior to the test.
- A zero time (baseline) blood sample is drawn. (fasting sample)
- The patient is then given a measured dose of glucose solution (1 gram glucose / kg of body weight) to drink within a 5 minute time frame.
- Blood is drawn at intervals (each 30 minutes for 2-3 hours) for measurement of glucose (blood and urine sugar), and sometimes insulin levels.

Note: *Usually the OGTT is performed in the morning as glucose tolerance can exhibit a diurnal rhythm with a significant decrease in the afternoon.*

Note: *The test should not be performed in patients suffering from trauma, recovery from a serious illness.*

Note: *Drugs as corticosteroids and diuretics may impair glucose tolerance and should be stopped before the test if possible.*

Interpretation:

The glucose tolerance test are used to diagnose several blood sugar metabolism disorders.

- **Normal Minimum curve**

Time [hours]	0	0.5	1	2	3
bGmin [mg/dl]	80	90	105	90	80

A "Normal-Min" curve means your pancreas is still in very good shape. Insulin release is strong and sufficient to keep glucose from rising.

- **Normal Maximum curve**

Time [hours]	0	0.5	1	2	3
bGmax [mg/dl]	120	135	160	130	110

The "Normal-Max" response means you are already well started on the road to diabetes. This is "normal" but it is not good. You are aging faster than the minimum possible. Your pancreas is still releasing enough insulin, but maybe 10% to 30% of your beta cells are not functioning. The stream of insulin is not as much as it was before which is the reason why glucose is rising.

❖ **Mild diabetes curve**

Time [hours]	0	0.5	1	2	3
bG [mg/dl]	115	145	180	160	120

Your pancreas is already partly shut down... perhaps 40% to 60% of your beta cells are burned out, and the stream of insulin is not enough to lower glucose quickly. Your pancreas is working overtime to bring down glucose. For this reason you are burning beta cells at a faster rate than ever before. The low quality of the insulin is not as good as before, and the long time that insulin is present to bring down your glucose causes you to become resistant to insulin.
This curve may be seen in hyperactivity of thyroid gland or pituitary and in severe infection

❖ **Severe diabetes curve**

Time [hours]	0	0.5	1	2	3
bG [mg/dl]	200	235	265	280	300

❖ **Pre-hypoglycemia curve**

Time [hours]	0	0.5	1	2	3
bG [mg/dl]	90	115	140	100	85

The curve is typical for a pre-stage of hypoglycemia. However, a range of mild symptoms may be present at this stage.

❖ **Mild hypoglycemia curve**

Time [hours]	0	0.5	1	2	3
bG [mg/dl]	80	120	80	60	80

This curve represents a mild form of hypoglycemia. Within the hour, the bG level drops to normal value. During the second hour, the value is far too low, this is typical in case of reactive hypoglycemia. Consequently, the curve rises until the normal value are reached. Because the bG level drops 40 (mg/dl) within half an hour, severe symptoms may occur.

❖ **Continuous low values curve**

Time [hours]	0	0.5	1	2	3
bG [mg/dl]	60	80	100	60	60

The curve stays under normal levels during the entire test and . A rare tumor, called insulinomia, may be the cause..

- **Severe hypoglycemia curve**

Time [hours]	0.5	1	2	3	4	5
bG [mg/dl]	100	170	110	130	170	125

This 'sawtooth-curve' indicates a severe form of hypoglycemia, though values do not actually drop below normal values. However, severe symptoms may occur.

- **Flat curve**

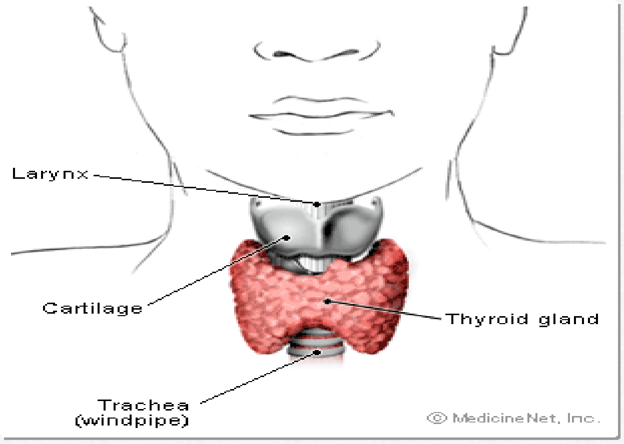
Time [hours]	0	0.5	1	2	3
bG [mg/dl]	90	90	90	100	90

This is a flattened glucose tolerance curve where blood glucose level fails to rise normally after glucose load. This curve may be due to malabsorption, hypopituitarism, insulinoma and Addison's disease.

Thyroid function test

Thyroid Gland

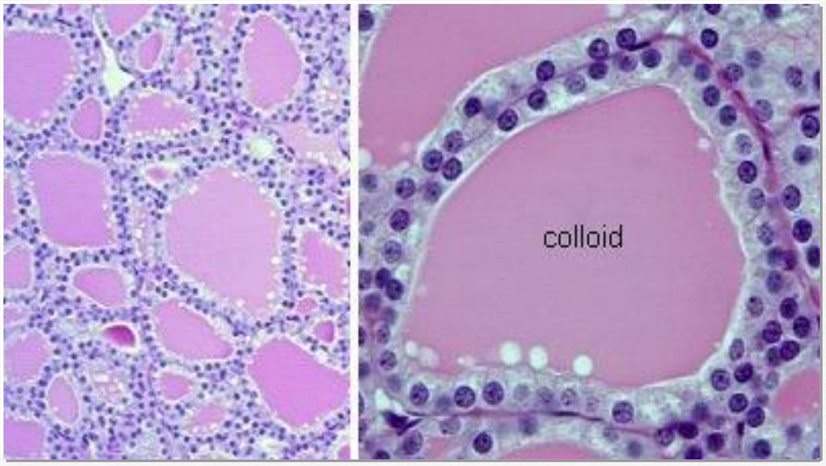
The thyroid is a small gland, measuring about 2 inches across, that lies just under the skin below the Adam's apple in the neck. The two halves (lobes) of the gland are connected in the middle (called the isthmus), giving the thyroid gland the shape of a butterfly



Close examination of a thyroid gland will reveal one or more small, light-colored nodules on or protruding from its surface - these are parathyroid glands (meaning "beside the thyroid").

Microscopic structure

Thyroid epithelial cells - the cells responsible for synthesis of thyroid hormones - are arranged in spheres called thyroid follicles.
Follicles are filled with colloid, a proteinaceous depot of thyroid hormone precursor.
In addition to thyroid epithelial cells, the thyroid gland houses one other important endocrine cell nestled in spaces between thyroid follicles are ***parafollicular or C cells***, which secrete the hormone calcitonin.



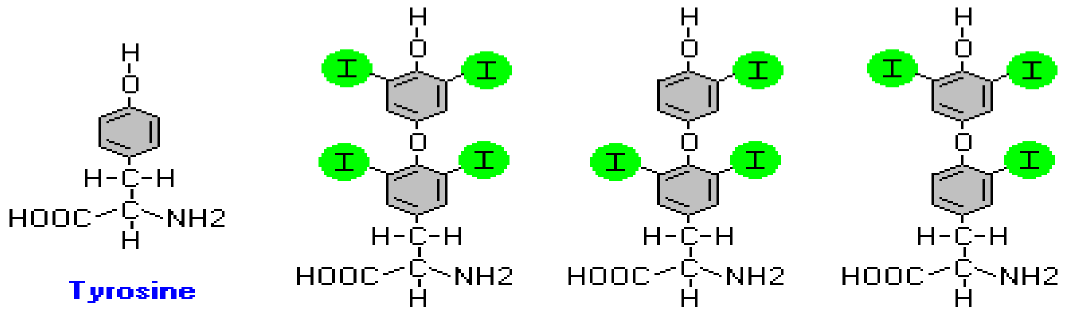
Thyroid hormones

The two thyroid hormones are T4 (thyroxine) and T3 (triiodothyronine). T4, the major hormone produced by the thyroid gland.

Chemistry of Thyroid Hormones

Thyroid hormones are derivatives of the amino acid tyrosine bound covalently to iodine. The two principal thyroid hormones are:

- thyroxine (also known as T4 or L-3,5,3',5'-tetraiodothyronine)
- triiodotyronine (T3 or L-3,5,3'-triiodothyronine)



Tyrosine

Thyroxine (T4)

Triiodothyronine (T3)

"Reverse T3" (inactive)

Synthesis of Thyroid Hormones

The entire synthetic process occurs in three major steps:

- Production and accumulation of the raw materials
- synthesis of the hormones on a backbone or scaffold of precursor
- Release of the free hormones from the scaffold and secretion into blood

The recipe for making thyroid hormones calls for two principle raw materials:

1. ***Tyrosines*** are provided from a large glycoprotein scaffold called *thyroglobulin*, which is synthesized by thyroid epithelial cells and secreted into the lumen of the follicle. A molecule of thyroglobulin contains 134 tyrosines, although only a handful of these are actually used to synthesize T4 and T3.
2. ***Iodine***, or more accurately iodide (I^-), is avidly taken up from blood by thyroid epithelial cells.

Fabrication of thyroid hormones is conducted by the enzyme thyroid peroxidase, an integral membrane protein present in the plasma membrane of thyroid epithelial cells. Thyroid peroxidase catalyzes two sequential reactions:

1. Iodination of tyrosines on thyroglobulin (also known as "organification of iodide").
2. Synthesis of thyroxine or triiodothyronine from two iodotyrosines.

Through the action of thyroid peroxidase, thyroid hormones accumulate in colloid, on the surface of thyroid epithelial cells. Remember that hormone is still tied up in molecules of thyroglobulin - the task remaining is to liberate it from the scaffold and secrete free hormone into blood.

Thyroid hormones are excised from their thyroglobulin scaffold by digestion in lysosomes of thyroid epithelial cells which contain hydrolytic enzymes that digest thyroglobluin, thereby liberating free thyroid hormones.

Finally, free thyroid hormones apparently diffuse out of lysosomes, through the basal plasma membrane of the cell, and into blood where they quickly bind to carrier proteins for transport to target cells.

> **Note:** *Thyroid hormones are poorly soluble in water, and more than 99% of the T3 and T4 circulating in blood is bound to carrier proteins. The principle carrier of thyroid hormones is thyroxine-binding globulin, a glycoprotein synthesized in the liver.*

> **Note:** *large majority hormone secreted from thyroid gland are T4, but the more active hormone T3 is derived by deiodination in peripheral tissues, especially liver and kidney.*

Control of Thyroid Hormone Synthesis

The thyroid gland is part of the hypothalamic-pituitary-thyroid axis, and control of thyroid hormone secretion is exerted by classical negative feedback, as depicted in the diagram.

- Thyroid-releasing hormone (TRH) from the hypothalamus stimulates TSH from the pituitary, which stimulates thyroid hormone release.
- The chief stimulator of thyroid hormone synthesis is thyroid-stimulating hormone from the anterior pituitary. Binding of TSH to receptors on thyroid epithelial cells seems to enhance all of the processes necessary for synthesis of thyroid hormones, including synthesis of the iodide transporter, thyroid peroxidase and thyroglobulin.

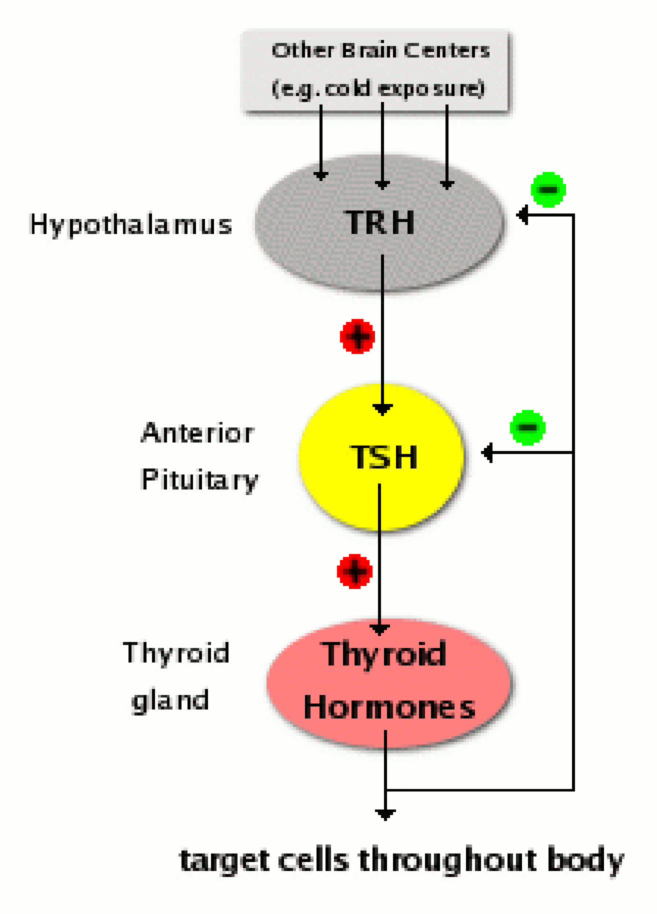
As blood concentrations of thyroid hormones increase, they inhibit both TSH and TRH, leading to "shutdown" of thyroid epithelial cells. Later, when blood levels of thyroid hormone have decayed, the negative feedback signal fades, and the system wakes up again.

Physiologic Effects of Thyroid Hormones

It is likely that all cells in the body are targets for thyroid hormones. While not strictly necessary for life, thyroid hormones have profound effects on many "big time" physiologic processes, such as development, growth and metabolism.

Thyroid hormones stimulate diverse metabolic activities in most tissues, leading to an increase in basal metabolic rate. One consequence of this activity is to increase body heat production, which seems to result, at least in part, from increased oxygen consumption and rates of ATP hydrolysis.

A few examples of specific metabolic effects of thyroid hormones include:



- ***Lipid metabolism***: Increased thyroid hormone levels stimulate fat mobilization, leading to increased concentrations of fatty acids in plasma. They also enhance oxidation of fatty acids in many tissues. Finally, plasma concentrations of cholesterol and triglycerides are inversely correlated with thyroid hormone levels - one diagnostic indication of hypothyroidism is increased blood cholesterol concentration.
- ***Carbohydrate metabolism***: Thyroid hormones stimulate almost all aspects of carbohydrate metabolism, including enhancement of insulin-dependent entry of glucose into cells and increased gluconeogenesis and glycogenolysis to generate free glucose.
- ***Growth***: Thyroid hormones are clearly necessary for normal growth in children and young animals, as evidenced by the growth-retardation observed in thyroid deficiency.

Common Thyroid Problems

Hyperthyroidism

What is hyperthyroidism?

Hyperthyroidism is a condition in which an overactive thyroid gland is producing an excessive amount of thyroid hormones that circulate in the blood. ("Hyper" means "over" in Greek). ***Thyrotoxicosis*** is a toxic condition that is caused by an excess of thyroid hormones from any cause.

What are the symptoms of hyperthyroidism?

Hyperthyroidism is suggested by **several** signs and symptoms; however, patients with mild disease usually experience no symptoms. In general, the symptoms become more obvious as the degree of hyperthyroidism increases. The symptoms usually are related to an increase in the metabolic rate of the body. Common symptoms include:

- *Palpitations*
- *Heat intolerance*
- *Nervousness*

- *Insomnia*
- *Breathlessness*
- *Increased bowel movements*
- *Light or absent menstrual periods*
- *Fatigue*
- *Fast heart rate*
- *Trembling hands*
- *Weight loss*
- *Muscle weakness*
- *Warm moist skin*
- *Hair loss*

Graves' Disease

Graves' disease, which is caused by a generalized overactivity of the thyroid gland, is the most common cause of hyperthyroidism. In this condition, the thyroid gland usually is renegade, which means it has lost the ability to respond to the normal control by the pituitary gland via TSH.

Graves' disease is thought to be an autoimmune disease, and antibodies that are characteristic of the illness may be found in the blood. These antibodies include thyroid stimulating immunoglobulin (TSI antibodies), thyroid peroxidase antibodies (TPO), and TSH receptor antibodies. The triggers for this disease include stress, smoking, and infectious organisms such as viruses.

Graves disease may be associated with eye disease (Graves' ophthalmopathy). Ophthalmopathy can occur before, after, or at the same time as the hyperthyroidism. Early on, it may cause sensitivity to light and a feeling of "sand in the eyes." The eyes may protrude and double vision can occur. The degree of ophthalmopathy is worsened in those who smoke.

De Quervain's Thyroiditis

De Quervain's Thyroiditis (also called ***subacute or granulomatous thyroiditis***) was first described in 1904 and is much less common than Hashimoto's Thyroiditis.

Symptomes

The thyroid gland generally swells rapidly and is very painful and tender.
The gland discharges thyroid hormone into the blood and the patients become hyperthyroid
Patients frequently become ill with fever.

Thyroid antibodies are not present in the blood, but the sedimentation rate, which measures inflammation, is very high.

Although this type of thyroiditis resembles an infection within the thyroid gland, no infectious agent has ever been identified and antibiotics are of no use.

Treatment

Treatment is usually bed rest and aspirin to reduce inflammation.
Occasionally cortisone (steroids) (to reduce inflammation) and thyroid hormone (to "rest" the thyroid gland) may be used in prolonged cases.

Hypothyroidism

Hypothyroidism is a condition in which the body lacks sufficient thyroid hormone. Since the main purpose of thyroid hormone is to "run the body's metabolism", it is understandable that people with this condition will have symptoms associated with a slow metabolism.

- ***Classification:*** It is often classified by the organ of origin

Type	Origin	Description
Primary hypothyroidism	thyroid gland	The most common forms and it may caused by Hashimoto's thyroiditis (an autoimmune disease) or Iodine deficiency (the most common cause of hypothyroidism)
Secondary hypothyroidism	pituitary gland	Occurs if the pituitary gland does not create enough thyroid stimulating hormone (TSH) to induce the thyroid gland. it is usually caused when the pituitary is damaged by a tumor, radiation, or surgery.
Tertiary hypothyroidism,	hypothalamus	Results when the hypothalamus fails to produce sufficient TRH.

- ***Symptoms of Hypothyroidism***

- *Weakness*
- *Weight gain or increased difficulty losing weight*
- *Coarse, dry hair*
- *Dry, rough pale skin*
- *Hair loss*
- *Cold intolerance (can't tolerate the cold like those around you)*
- *Muscle cramps and frequent muscle aches*
- *Constipation*
- *Depression*
- *Irritability*
- *Memory loss*
- *Abnormal menstrual cycles*

Hashimoto's Thyroiditis

Hashimoto's Thyroiditis (also called ***autoimmune or chronic lymphocytic thyroiditis***) is the most common type of thyroiditis.
The thyroid gland is always enlarged, although only one side may be enlarged enough to feel. During the course of this disease, the cells of the thyroid becomes inefficient in converting iodine into thyroid hormone and "compensates" by enlarging.

- ***Clinical signs***

the patient is hypothyroid because the gland retains the ability to take-up or "trap" iodine even after it has lost its ability to produce thyroid hormone. As the disease progresses, the TSH increases since the pituitary is trying to induce the thyroid to make more hormone, the T4 falls since the thyroid can't make it, and the patient becomes hypothyroid.
Thyroid antibodies are present in 95% of patients with Hashimoto's Thyroiditis and serve as a useful "marker" in identifying the disease without thyroid biopsy or surgery.

- ***Treatment***

is to start thyroid hormone replacement. This prevents or corrects the hypothyroidism and it also generally keeps the gland from getting larger.

Congenital hypothyroidism (cretinism)

Cretinism is a condition of severely stunted physical and mental growth due to untreated congenital deficiency of thyroid hormones (congenital hypothyroidism) due to maternal nutritional deficiency of iodine.

Approximately 1 in 4000 newborn infants has a severe deficiency of thyroid function, while even more have mild or partial degrees.

Symptoms

Infants born with congenital hypothyroidism may show no effects, or may display mild effects that often go unrecognized as a problem:

- excessive sleeping
- reduced interest in nursing
- poor muscle tone
- low or hoarse cry
- infrequent bowel movements
- exaggerated jaundice
- low body temperature

If untreated for several months after birth, severe congenital hypothyroidism can lead to growth failure and permanent mental retardation.

Treatment

Treatment consists of a daily dose of thyroid hormone (thyroxine) by mouth. Because the treatment is simple, effective, and inexpensive, nearly all of the developed world practices newborn screening to detect and treat congenital hypothyroidism in the first weeks of life.

Goiter

A goiter is an enlargement of the thyroid gland. It is not cancer.

Types and causes

There are different kinds of goiters:

Simple goiter:

usually occurs when the thyroid gland is not able to produce enough thyroid hormone to meet the body's needs (***Hypothyroidism***). The thyroid gland makes up for this lack by enlarging, which usually overcomes mild deficiencies of thyroid hormone.

A simple goiter may be classified as:

Endemic goiter:

Endemic goiters occur within groups of people living in geographical areas with iodine-depleted soil, usually regions away from the sea coast.

Sporadic goiter:

In most cases of sporadic goiter the cause is unknown. Occasionally, certain medications such as lithium can cause goiter.

Toxic goitre

It refers to goitre with ***hyperthyroidism***. These are most commonly due to Graves' disease, but can be caused by inflammation.

- ***Symptoms***
  - Thyroid enlargement varying from a single small nodule to massive enlargement (neck lump)
  - Breathing difficulties, cough, or wheezing due to compression of the windpipe
  - Swallowing difficulties due to compression of the esophagus
  - Neck vein distention and dizziness when the arms are raised above the head
- ***Treatment***

  A goiter only needs to be treated if it is causing symptoms. The enlarged thyroid can be treated with radioactive iodine to shrink the gland or with surgical removal of part or all of the gland (thyroidectomy). Small doses of iodine may help when the goiter is due to iodine deficiency.

Thyroid function test

- ***TSH test***
- Determine whether the thyroid gland is functioning properly.
- Determine the cause of an underactive thyroid gland (hypothyroidism).

TSH levels can help determine whether hypothyroidism is due to a damaged thyroid gland or some other cause (such as a problem with the pituitary gland or the hypothalamus).

- Confirm the diagnosis of an underactive thyroid gland in a newborn (congenital hypothyroidism).
- Monitor treatment with thyroid replacement medications for people who have hypothyroidism.
- Monitor thyroid gland function in people who are being treated for hyperthyroidism with antithyroid medications, surgery, or radiation therapy.

- ***T4 test***
- The total T4 test has been used for many years to help diagnose hyper- and hypothyroidism, it is usually ordered along with or following a TSH test.

Disadvantage

It is a useful test but can be affected by the amount of protein available in the blood to bind to the hormone.

For example, the increase in total levels associated with pregnancy, oral contraceptives and estrogen therapy

Free T4

The free T4 test is a newer test that is not affected by protein levels. Since free T4 is the active form of thyroxine, it is thought by many to be a more accurate reflection of thyroid hormone function and, in most cases, its use has replaced that of the total T4.

- ***T3 test***
- The T3 test is usually ordered following an abnormal TSH and T4 test. Either the total T3 or the free T3 may be ordered.

The following table summarizes test results and their potential meaning.

TSH	T4	T3	Interpretation
High	Normal	Normal	Mild (subclinical) hypothyroidism
High	Low	Normal or Low	Hypothyroidism in early stage Hypothyroidism
Low	Normal	Normal	Mild (subclinical) hyperthyroidism
Low	High or normal	High or normal	Hyperthyroidism
Low	Low or normal	Low or normal	Rare pituitary (secondary) hypothyroidism

Tumor markers

Tumour: (from Latin tumor "swelling") A tumor is a self controlling growth formed by the unlimited multiplication of abnormal cells in one of the body tissues or organs.

Tumor pathology

General Characters of Tumors

- The tumor forms a mass which has no useful function.
- The tumor does not obey the biologic factors which control physiological growth , its growth is unlimited ,and if the stimulus of growth stops or is removed, the tumor continues to grow. This independent growth is called (autonomous growth). autonomy.
- Any tissue may be the seat of tumor formation.
- The activity of the tumor is spent mainly in multiplication.

Classification of tumors

The most important subdivision of neoplasms is into those which are benign and those which are malignant. This distinctions is often made on the basis of histological criteria but it in an essentially clinical subdivision.

Benign tumor

Defnation

The term "benign" implies a mild and nonprogressive disease. A benign tumor is a tumor that lacks all three of the malignant properties of a cancer. Thus, by definition, a benign tumor:

- does not grow in an unlimited, **aggressive** manner
- does not **invade** surrounding tissues
- does not **metastasize**

Characters of benign tumors

Gross Picture:

Benign tumors are ***capsulated.*** usually of small size. in a solid organ takes ***a spherical or ovoid shape***

Microscopic Picture:

- The cells of a benign tumor resemble the cells of the tissue of origin (***differentiation).***
- The cells are usually small and of nearly equal size and similar shape.
- Benign tumors have few blood vessels.

Rate of growth: Slow.

Mode of growth: Grow by ***expansion***, i.e. compresses the surrounding tissue to form room for itself.

Localization: Localized at the site of origin. They do not spread.

Recurrence: Absent if well excised, so the prognosis is good.

Malignant tumor

Deflation

by definition, a malignant tumor:

- grow in an unlimited, **aggressive** manner
- **invade** surrounding tissues
- spread or **metastasis** of cancerous cells

Characters of malignant tumors

Gross Picture:

Malignant tumors are not capsulated tumors usually reach large size in short time and take different gross shapes according to the organ.

Microscopic Picture:

Malignant tumors fail to reproduce the exact appearance and pattern of the parent tissue i.e. they show ***loss of differentiation.***

Malignant tumors showing great similarity to parent tissue are called "well differentiated", moderate similarity "moderately differentiated", no similarity "undifferentiated".

Tumor angiogenesis:

This process of forming new blood vessels is called angiogenesis. Tumor angiogenesis is the proliferation of a network of blood vessels that penetrates into cancerous growths, supplying nutrients and oxygen and removing waste products and also required for the spread of a tumor.

Rate of growth: Rapid.

Mode of growth:

Infiltrating mode i.e. the malignant cells proliferate and push themselves in-between the surrounding normal cells in all directions, and take most of their nutrition, so the normal cells undergo necrosis and give their place to the malignant cells.

Localization:

The tumor does not remain locally at the site of origin, but sends secondaries or metastasis to other organs.

Recurrence:

Surgical removal is often followed by recurrence. The cause of recurrence is:

(a) From tumor cells left over at the operation site.
(b) A new start near by the first tumor.

Prognosis in malignant tumors

The prognosis of malignant tumors depends upon:

- ***Type of the tumor:*** Certain malignant tumors are more aggressive than others e.g. malignant melanoma has a poor prognosis than squamous cells carcinoma.
- ***Tumor stage:*** The extent of spread and the presence or absence of metastasis are the most important factors in prognosis.
- ***Tumor grade:*** Well differentiated tumors are usually of better prognosis.
- ***Immune responses:*** Cellular immunity is believed to play a role ii the defense mechanism against tumors through cytotoxic T-cells

Classification of malignant tumors

Carcinoma

carcinoma is any cancer that arises from epithelial cells (squamous, glandular , …) . It is malignant by definition , thus it is the malignant tumor of non-mesodermal tissues.

General features

- More common than sarcomas.
- Occurs in middle and old age (40-60 years).
- Slower in growth than sarcoma.
- It grows more by infiltration than by expansion.
- Does not form a bulky mass.
- Consistency is usually hard

Sarcoma

A sarcoma is a cancer of the connective or supportive tissue (bone, cartilage, fat, muscle, blood vessels) and soft tissue.

General features

- These tumors form large or bulky masses .
- Sarcoma grows rapidly and is not capsulated. Therefore its limits are ill-defined from the surrounding tissues.

- It grow by expansion and by infiltration.
- It occurs in younger age groups — in adolescents and young adults (10-20 years).
- These tumors are cellular tumors and that is why they are soft in consistency
- These tumors are vascular tumors. The blood vessels are thin walled.

Difference between carcinoma and sarcoma

Characters	carcinoma	sarcoma
Incidence	More common	Less common
Age	middle and old age	adolescents and young adults
Rate of growth	slower growth	Rapid growth
Mode of growth	expansion and by infiltration	expansion and by infiltration
Spread	slower	faster
Gross picture	Does not form a bulky mass	form large or bulky masses
consistency	usually hard	soft
Microscopic picture	cells are arranged in groups	cells do not collect in groups

Cancer Metastasis

Metastasis it is the spread of a cancer from one organ or part to another non-contiguous organ or part. Only malignant tumor cells have the capacity to metastasize.

Metastatic tumor

When cancer cells spread to form a new tumor, it is called a secondary, or metastatic tumor, and its cells are like those in the original tumor. This means, for example, that if breast cancer spreads (metastasizes) to the lung, the secondary tumor is made up of abnormal breast cells (not abnormal lung cells). The disease in the lung is then called metastatic breast cancer (not lung cancer).

Routes of spread

(1) Direct or Local Spread *(Infiltration of tissue spaces)*

Malignant cells **Infiltrate** surrounding tissues in all directions along the lines of least resistance .

e.g. : bone cancer

- This is facilitate by the fact that cancer cells are amoeboid and motile.
- Tumor cells are less adherent to each other than normal cells and so they can be easily separated from each other.

(2) Distant Spread

(1) Lymphatic Spread:

The malignant cells invade the wall of a lymph Vessel ,detach as small groups and are carried by the current of lymph as tumor emboli.

The emboli reach the draining lymph node by the afferent lymphatics.

e.g. : Cancer of breast spreads to axillary lymph nodes.

(2) Blood Spread: (*Hematogenous spread)*

(a) Malignant cells invade the thin walled veins, become detached and pass in the blood as tumor emboli This commonly occurs with sarcomas.

carcinomas spreading by lymphatics and reach the thoracic duct will give rise to tumor emboli in blood.

Carcinogenesis

Carcinogenesis (meaning literally, the creation of cancer) is the process by which normal cells are transformed into cancer cells.

Carcinogenesis is caused by this mutation of the genetic material of normal cells, which upsets the normal balance between proliferation and cell death , which results in uncontrolled cell division and tumor formation.

Only mutations in those certain types of genes which play vital roles in cell division, apoptosis (cell death), and DNA repair will cause a cell to lose control of its cell proliferation.

Carcinogen

The term carcinogen refers to any substance, microbe or radiation which is an agent directly involved in the promotion of cancer or in the facilitation of its propagation.

❖ Chemical Carcinogens

Chemical carcinogenesis consists of a multiple-phase phenomenon in which chemicals initially damage DNA and then other chemicals or environmental cofactors cause abnormal cells to proliferate.

Cellular oncogenes and tumor suppressor genes are two critical DNA targets for chemical carcinogens, leading to the activation of oncogenes and the inactivation of suppressor genes .

Types of chemical carcinogens :

Ultimate carcinogens

Primary or ultimate carcinogens are those that are biologically active because of their chemical structure. These are usually electrophilic reagents that interact directly and specifically with cellular macromolecules forming a covalent addition product (i.e., adduct) with cellular macromolecules, including DNA.

Examples: alkylating agents such as nitrogen mustards.

pro-carcinogens

Secondary or pro-carcinogens, which themselves are inert, must first be activated to ultimate carcinogens by host-mediated enzymatic reactions

After the pro-carcinogens enters the body, the body makes an attempt to eliminate it through a process called biotransformation. The purpose of these reactions is to make the carcinogen more water-soluble so that it can be removed from the body. But these reactions also convert a less toxic carcinogen into a more toxic one.

Examples

polycyclic aromatic hydrocarbons (PAHs)

Many PAHs behave as procarcinogens (as benzpyrene that is present in coal tar and cigarette). They do not cause cancer per se, but they can be converted to active carcinogens by enzymes located in organs like the liver and lung.

Aflatoxin B1

The fungus *Aspergillus flavus* produces the polar polycyclic compounds Aflatoxin B1 which is hepatotoxic and carcinogenic. Aflatoxins are activated by cytochrome P-450 enzymes .

co-carcinogens

A third category of compounds, which apparently are not directly carcinogenic, but they are co-carcinogens. They promote cancer induction by potentiating the effects of ultimate or pro-carcinogens.

Examples

Among of the best-known co-carcinogens are phorbols, and their esters. The most common phorbol ester is phorbol-12-myristate-13-acetate (PMA), which is used as biomedical research tool in models of carcinogenesis.

❖ Physical carcinogens

Radiation is suggested to cause two percent of all cancer deaths. Most of these fatalities result from exposure to natural sources of radiation.

Types of radiations

- **Particulate ionizing radiations :** e.g. Alpha particles, beta particles, neutrons and protons. Many cases of leukemia developed as a late result of the atomic radiations in Hiroshima and Nagazaki after the second world war.
- **Ultraviolet radiation (UV light) :** Ultraviolet radiation is a relatively low-energy emission and does not penetrate deeply. Thus, skin absorbs most of the ultraviolet radiation and it is the location of most ultraviolet irradiation tumors.
- **high-energy radiation** e.g. X ray and gamma rays. It have sufficiently high energy to disrupt electrons from atoms.

❖ Biological carcinogens

Viral carcinoges

More recently, it has emerged that some human cancers, particularly epithelial cancers, are caused by virus infection. What happens is that the virus can cause genetic changes in cells that make them more likely to become cancerous. But many people can be infected with a cancer-causing virus, and never get cancer. Thus virus only causes cancer in certain situations.

How viruses are thought to cause cancer ?

This may occur through the integration of viral genetic elements into the host DNA. These new genes may be expressed by the host; they may affect cell growth or division or disrupt normal host genes required for control of cell growth and division.

Examples of cancer-causing virus

- ***Human papillomaviruses (HPVs):*** HPV infection is the main cause of cervical cancer. It also may be a risk factor for other types of cancer.
- ***Hepatitis B and hepatitis C viruses:*** Liver cancer (Hepatocellular carcinoma) can develop after many years of infection with hepatitis B or hepatitis C.

There will be people with primary liver cancer who haven't had the related virus. But infection may increase their risk of getting that particular cancer.

Bacterial carcinoges

In addition to viruses, researchers have noted a connection between bacteria and certain cancers.

- ***Helicobacter pylori :*** This bacterium can cause stomach ulcers. It also can cause stomach cancer and lymphoma (non-Hodgkin's lymphoma) in the stomach lining.

Parasitic carcinoges

- ***Schistosoma haematobium*** causes chronic inflammation and fibrosis of the bladder, which may lead to bladder cancer.

Tumor marker

Tumor markers are substances, usually proteins, that are produced by the body in response to cancer growth or by the cancer tissue itself and that may be detected in blood, urine, or tissue samples.
Some tumor markers are specific for a particular type of cancer, while others are seen in several cancer types. Most of the well-known markers may also be elevated in non-cancerous conditions. Consequently, tumor markers alone are not diagnostic for cancer.

❖ Why are they done?

Tumor markers are not diagnostic in themselves. A definitive diagnosis of cancer is made by looking at tissue biopsy specimens under a microscope. However, tumor markers provide information that can be used to:

- **Screen**. Most markers are not suited for general screening, but some may be used in people with a strong family history of a particular cancer. As mentioned, PSA testing may be used to screen for prostate cancer.
- **Diagnose**. In a person who has symptoms, tumor markers may be used to help identify the source of the cancer, such as CA-125 for ovarian cancer, and to help differentiate it from other conditions. Remember that tumor markers cannot diagnose cancer by themselves but aid in this process.
- **Stage**. If a person does have cancer, tumor marker elevations can be used to help determine how far the cancer has spread into other tissues and organs.
- **Determine Prognosis**. Some tumor markers can be used to help doctors determine how aggressive a cancer is likely to be.
- **Monitor Treatment**. Tumor markers can be used to monitor the effectiveness of treatment, especially in advanced cancers. If the marker level drops, the treatment is working; if it stays elevated, adjustments are needed.
- **Determine Recurrence**. Currently, one of the most important uses for tumor markers is to monitor for cancer recurrence. If a tumor marker is elevated before treatment, low after treatment, and then begins to rise over time, then it is likely that the cancer is returning. (If it remains elevated after surgery, then chances are that not all of the cancer was removed.)

Types of tumor marker

Biochemical markers produced by cancers include:

Enzymes

Enzymes are released from cells into the systematic circulation as the result of tumor necrosis or the change of membrane permeability of the cancer cell.
Most enzymes are not unique for a specific organ. Therefore, enzymes are most suitable as nonspecific tumor marker.

Some of enzymes used as tumor markers:

Enzymes	Type of cancer
• Alkaline phosphatase	• Bone, leukemia
• Amylase	• Pancreas
• γ-Glutamyltransferase	• Liver
• Lactate dehydrogenase	• Liver, lymphomas, leukemia
• Prostatic acid phosphatase	• Prostate
• Neuron specific enolase (NSE)	• neuroblastoma

Hormones

The production of hormones in cancer involves:

1. Endocrine tissue that produce excess amounts of a hormones.
2. A hormone produced by no endocrine tissue (ectopic syndrome).

Elevation of a given hormone is not diagnostic of a specific tumor, as a hormone may produce by a variation of cancers.

Some of hormones used as tumor markers.

Hormone	Type of cancer
• Calcitonin • Human chorionic gonadotropin (hCG) • Prolactin	• Medullary thyroid • Choriocarcinoma, testicular cancer • Pituitary adenoma

Oncofetal Antigen

Oncofetal antigens are glycoproteins produced during fetal life.
These proteins are present in high concentration in the sera of fetuses only and decrease to low levels or disappear after birth. But in cancer patients, these proteins are existed in large amount.

Some of oncofetal proteins used as tumor marker:

Name	Type of cancer
• α-Fetoprotein • carcinoembryonic antigen	• Hepatocellular carcinoma • colon cancer

Note: *In healthy adults, the serum AFP level is less than 10 μg /L. In addition to pregnancy, elevated levels of serum AFP are associated with benign liver conditions such as hepatitis and cirrhosis.*
Note: *CEA levels are elevated in some patients with benign conditions such as cirrhosis.*

Carbohydrates antigens

Carbohydrate-related tumor markers either are antigens on the tumor cell surface or are secreted by the tumor cells.
They tend to be more specific than naturally secreted markers.

Some of Carbohydrates antigens used as tumor marker.

Name	Type of cancer
• CA 15.3 • CA 19.9 • CA 125	• Breast, ovarian, pancreatic, lung, colorectal • pancreas • Ovarian

Proteins

several proteins have been proposed to be used as tumor markers as in the following table.

Name	Type of cancer
• C-peptide • Ferritin • Bence jonse protein • B2M (Beta-2 microglobulin) • PSA (Prostate specific antigen)	• Insulinoma • Liver, lung, breast, leukemia • Multiple myeloma, • Multiple myeloma, and lymphomas • Prostatic cancer

Genetic markers

Multiple genetic alterations may be necessary for the transformation of a cell from a normal state to a cancerous one and, finally, for metastasis spread.

Two classes of gene are implicated in the development of cancer:

1. Oncogenes.
2. Tumor suppressor genes.

Oncogenes

Oncogenes are activated by dominant mutations, and associated with hematological malignancies such as leukemia.

Oncogenes are involved in growth factor signaling pathways, thus amplification of the oncogene leads to abnormal cell growth.

The level of expression in tissue appears to correlate with the stage or grade of the tumor.

Oncogene markers:

Oncogene	Function	Type of cancer
N-ras-mutation	Signal transduction	Acute myeloid leukemia, neuroblastoma
c-myc translocation	Transcription regulation	B and T cell lymphoma, lung cells
c-abl/bcr translocation	Signal transduction	Chronic myelocytic leukemia
N-myc amplification	Transcription regulation	neuroendocrine

Tumor suppressor genes

The suppressor genes have only been isolated from solid tumors.

The oncogenicity of suppressor genes is derived from loss rather than activation.

Any mutation either deletion in suppressor genes or alterations in chromosomes (monosomy) lead to high uncontrolled cell activity.

Suppressor gene markers.

Suppressor gene	Chromosome	Type of cancer
DCC (deleted colon carcinoma)	18q	Colon, adenoma
RB (retinoblastoma)	13q	Eye
p53	17q	Various carcinoma

Note: *Mutations in p53 apparently allow cells to move through the cell cycle and contribute to the autonomous growth of cancer.*

Laboratory quality control

Quality management

Quality Assurance - QA is defined as the overall program that ensures that the final results reported by the laboratory are correct.

Quality Control - QC refers to the measures that must be included during each assay to verify that the test is working properly.

"The aim of quality control is simply to ensure that the results generated by the test are correct. However, quality assurance is concerned with much more: that the right test is carried out on the right specimen, and that the right result and right interpretation is delivered to the right person at the right time"

Types of quality control

Internal Quality Control (IQC): which denotes a set of procedures undertaken by the staff of the laboratory for continuously assessing laboratory work so that quality results are produced by the laboratory.

How IQC can be done?

This is can be done by using a sample of known concentration to assess the quality of instrument and reagent

What is a Control?

Controls, which are often purchased from manufacturers, use a human base to ensure the analytes being tested parallel human ranges.

Manufacturers pool together many human blood samples to create the large volume needed for a lot number of control. Examples of controls such as Zymotrol , Unitrol , Lyotrol

External Quality Control (EQC): is a system of objectively assessing the laboratory performance by an outside agency. This assessment is retrospective and periodic but is aimed at improving the IQC. Example , the American system CAP

Factors affecting laboratory quality

There are many pre-analytical and post-analytical factors which influence the quality of the end results to a very significant extent such as:

Specimen: This is the single most important factor. Selection of the right sample, collection in a right manner, adequate quantity, proper transportation to the laboratory, and processing of the sample before testing, are crucial factors.

Personnel: The quality of the laboratory results generated is directly proportional to the training, commitment and motivation of the technical staff.

Analytical factors: The quality of reagents, chemicals, glassware, stains, culture media,use of standard procedures and reliable equipment all influence laboratory results. Failure to examine a sufficient number of microscope fields can lead to false negative results.

Post analytical factors: Transcription errors, incomplete reports, and improper interpretation can adversely influence the laboratory results.

Sources of errors in laboratory practice

Laboratory personnel need to be aware of the errors that can occur when collecting specimens (pre-analytical stage), testing specimens (analytical stage), reporting and interpreting test results (post-analytical stage).

1. *Pre -analytical Laboratory Errors*

- اخذ اسم المريض خطأ او غير كامل
- قراءه التحليل خطأ مما يؤدى الى تحضير أنابيب خطأ لسحب العينة
- اخذ عينه التحليل بدون التأكد من الشروط الخاصة به مثل السائل المنوي أو الدهون
- تبديل عينات البول و البراز (لابد من كتابه اسم المريض على الكوب نفسه و ليس الغطاء)
- عدم مراعاة النسبة الصحيحة بين الدم و مانع التجلط
- سحب عينه دم متكسره (Hemolysis)
- التأخر فى سحب العينه مما يعرض الدم للتجلط داخل السرنجه
- -سحب عينه دم غير مكتمله خاصه فى التحاليل التى تتطلب كميه محدده من الدم مثل البروثرومبين
- اختيار خاطئ للمادة المانعة للتجلط مثلا سحب عينه اختبار التجلط على ايديتا
- عدم كتابه اسم المريض على الانبوبه المحتوية على الدم بعد سحب العينة
- تخزين العينة فى ظروف خطأ (مثلا درجه حرارة خطأ)
- كمية غير كافية من العينة لعمل الفحص.
- اخذ عينه خطأ لا تناسب التحليل مثلا اخذ عينه من الوريد لعمل تحليل غازات الدم
- عينه تم أخذها فى وقت غير ملائم مثل سحب عينه هرمون الكورتيزول في غير موعده المحدد

ملحوظة: لابد من التأكد من سلامه وصلاحية عينه الدم قبل مغادره المريض المعمل

2. *Analytical Laboratory Errors*

- خطأ في اختيار الطول الموجى أو الفلتر المناسب للتحليل
- خطأ في برمجه الاجهزه يؤدى لخطأ في النتيجة
- اختيار جهاز خطأ أو طريقه خطأ أو غير دقيقه لإجراء التحليل
- تذبذب القراءة نتيجة لعدم ثبات التيار الكهربائي مما يؤدى إلى نتائج خاطئة
- عدم المحافظة على درجة الحرارة المناسبة أثناء التحليل
- عدم الالتزام بالوقت المطلوب للتحليل
- تخفيف العينات و عدم حساب التخفيف في النتيجة النهائية
- عدم الدقة في التعامل مع الماصات و أدوات سحب السوائل (الأوتوماتك بايبت)
- استخدام أنابيب أو أدوات غير نظيفة أو غير جافه أثناء إجراء التحليل
- قراءه النتيجة في كوفيت ملوثه ببصمات الأصابع أو العينة محتويه على فقاعات هواء
- عدم إجراء تقييم لحاله الاجهزه كل فتره محدده (quality control)
- استخدام كيماويات منتهية الصلاحية

ملحوظة: لابد من الفحص المستمر للـ Reagent و انه ليس به تعكير أو ترسيب أو تغيير باللون و عدم الاعتماد فقط على تاريخ انتهاء الصلاحية

3. *Post -analytical Laboratory Errors*

- خطاء فى كتابه النتائج الرقمية (خاصة النتائج ذات الأرقام العشرية)
- خطأ فى كتابه المعدل الطبيعي للتحليل (و الذي يختلف حسب السن و الجنس)
- تبديل البيانات مكان بعضها خاصة في التحاليل الكاملة مثل البول الكامل و صوره الدم
- تدوين النتائج بوحدات خاطئة
- كتابه اسم المريض أو اسم الطبيب خطأ
- كتابه تعليق خاطئ على نتيجة التحليل

ملحوظة: لابد من مراجعه نتيجة التحليل بعد طباعته جيدا و قبل تسليمه للمريض (اسم المريض – اسم الطبيب – نتيجة التحليل – النسب الطبيعية - التعليقات)

ملحوظة: لابد من مراجعه تطابق النتيجة مع الحالة الإكلينيكية للمريض و توافقها مع باقي تحليلات المريض في نفس اليوم

ملحوظة: لابد من مقارنة نتائج المريض بنتائجه السابقة و خاصة تحليلات السيولة – الأدوية – و دلالات الأورام و الجلوكوز

Standard Operating Procedure

Each laboratory should have Standard Operating Procedure Manuals (SOPMs) which should include the following information about the laboratory:

1. Biosafety precautions
2. Collection, transport and storage of specimens
3. Criteria of rejection of samples
4. Processing of specimens
5. Maintenance of equipment
6. test manual
7. Recording of results
8. Reporting of results
9. Procedure of quality control
10. Reference ranges

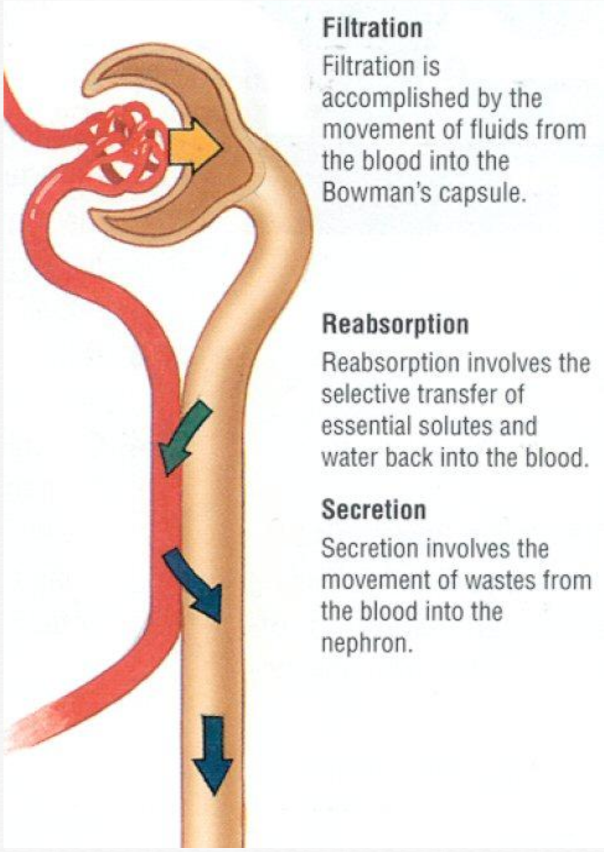
The preparation and implementation of SOPs will greatly assist in identifying, monitoring, and minimizing errors that lead to incorrect test results and the waste of resources.

Complete Urine analysis

Urine Formation

Urine is continuously formed by the kidneys. It is actually an ultrafiltrate of plasma from which glucose, amino acids, water, and other substances essential to body metabolism have been reabsorbed.

The kidney's ability to clear waste products selectively from the blood while maintaining the essential water and electrolyte balances in the body is controlled in the nephrons by:-



Glomerular filtration

The first step in urine formation is filtration of blood plasma at the nephrons.

In the nephrons cells, proteins, and other large molecules are filtered out of the glomerulus by a process of ultrafiltration, leaving an ultrafiltrate that resembles plasma (except that the ultrafiltrate has negligible plasma proteins) to enter Bowman's space.

Tubular reabsorption

In the proximal tubule, 60% to 80% of the ultrafiltrate is reabsorbed

Tubular reabsorption is the process by which solutes and water are removed from the tubular fluid and transported into the blood.

Note: *It is called reabsorption (and not absorption) because these substances have already been absorbed once (in the intestines).*

Tubular secretion

Tubular secretion is the transfer of materials from peritubular capillaries to renal tubular lumen.

Tubular secretion is caused mainly by active transport.

In the distal tubule, secretion is the prominent activity

Usually only a few substances are secreted. These substances are present in great excess, or are natural poisons.

Renal threshold

Certain substances appear in the urine when their plasma levels are above certain set-point, or "threshold," levels. High-threshold substances, such as glucose and amino acids, are reabsorbed almost completely. The appearance of a high-threshold substance in the urine is evidence that the filtered load of the substance is exceeding the maximal reabsorption rate of its transport system.

Complete urinalysis

Overview

A urinalysis is a group of tests that detect and semi-quantitatively measure various compounds that are eliminated in the urine, including the byproducts of normal and abnormal metabolism as well as cells, and cellular fragments.

Why urine analysis is done?

- As a general evaluation of health.
- To screen for a disease or infection of the urinary tract.
- To monitor the treatment of certain conditions such as kidney stones, a urinary tract infection (UTI), or some types of kidney or liver disease.
- Diagnosis of some metabolic and endocrine disturbances in the body such as diabetes.

Symptoms and conditions that required urine test

- Discolored or foul-smelling urine
- Pain during urination
- Blood in the urine (hematuria)
- Frequent urination
- Abdominal pain, or back pain
- Pregnant women to chick the risk of pregnancy toxemia

Collection of urine sample

Why the first voided morning specimen is preferred?

- Because it is usually more concentrated and therefore more likely to reveal abnormalities
- This is usually hypertonic and reflects the ability of the kidney to concentrate urine during dehydration which occurs overnight.

Precautions

- All samples should be midstream and collected in a clean sterile container.
- Wash your hands to make sure they are clean before collecting the urine.
- Avoid touching the inside or rim of the container with your fingers or genital area.

Collection method (midstream urine collection)

- Clean the area around your genitals.
- Begin urinating into the toilet.
- After the urine has flowed for several seconds, place the collection container into the stream and collect this “midstream” urine without interrupting the flow.
- Finish urinating into the toilet.
- Carefully replace the lid on the container and return it to the lab.

Note: *Collecting a urine sample from a small child or baby may be done by using a special plastic bag with tape around its opening, this bag is attached around the child's genitals until he or she urinates*

Note: *urine samples for bacteriological examination must be collected in clean sterilized container and culture done from separate sample or before routine examination*

Precautions for urine analysis

- The ideal situation is when the specimen is analyzed shortly after collection (within 1h)
- If examination can not be done directly after collection the sample must be refrigerated within 1 hour of collection.
- If the specimen is not refrigerated within 1 hour of collection, ***the following changes in composition may occur:***
  - Increased pH from the breakdown of urea to ammonia by urease-producing bacteria)
  - Decreased glucose from glycolysis and bacterial utilization
  - Decreased ketones because of volatilization
  - Decreased bilirubin from exposure to light
  - Increased bacteria from bacterial reproduction
  - Increased nitrite from bacterial reduction of nitrate
  - precipitation of amorphous urate
  - Changes in color caused by oxidation or reduction of metabolites
  - Increased turbidity caused by bacterial growth and precipitation of amorphous material
  - Disintegration of red blood cells (RBCs) and casts, particularly in dilute alkaline urine

Preservation of urine

Urine specimens should not remain un-refrigerated for longer than two hours. refrigeration will slow microbial growth and tends to stabilize most urine components for up to 12 hours

Note: *you must re-warm the sample before analysis.*

Urine sample rejection

Generally specimens that could lead to false interpretation should be rejected such as:

- The sample must be sufficient quantity { The widely accepted urine volume is 12 ml}
- Specimens that is contaminated from a woman's menstrual period.
- Not getting urine sample to lab in 2 hour.
- urine sample that is taken in dirty container
- Specimens that is contaminated with feaces

Note: *Before rejecting a specimen, we must consider that specimens may be unique and represent a punctual situation in time and the patient will have to come back in the case of a rejected specimen.*

Urine physical examination

Urine Volume

Overview:
Urine volume measurements are part of the assessment for fluid balance and kidney function. The normal volume of urine voided by the average adult in a 24-hour period ranges from 600 to 2500 ml; the typical amount is about 1200 ml. The amount voided over any period is directly related to the individual's fluid intake, the temperature and climate.

Procedure:
The total volume is recorded as urine volume in milliliters (cubic centimeters) per 24 hours.

> **Note:** *The volume of random urine sample may be measured using graduated container or it may be recorded as random sample in the report*

Interpretation:

Polyuria

polyuria is a condition characterized by the passage of large volumes of urine (at least 2.5 L over 24 hours in adults).

> **Note:** *Polyuria is sometimes used to refer to frequent urination, irrespective of the volume of urine passed.*

What are the causes of Polyuria?

Physiological causes

- Cold climate
- After drinking large amounts of fluids
- during pregnancy (frequent urination)
- High protein diet: end product is urea which cause osmotic diursis and decrease reabsorption of water .
- diuretic foods (foods and beverages containing caffeine, such as chocolate, coffee, tea, and soft drinks; hot spicy foods; juices high in acid; alcoholic beverages; etc.)

Pathological causes

- diabetes mellitus (glucose in urine cause osmotic diuresis)
- Diabetes inspidus (caused by a deficiency of antidiuretic hormone (ADH)
- hypercalcaemia (it leads to nephrogenic diabetes inspidus)
- Hyperparathyroidism (causing excessive mobilization of ca from bone so rise in plasma and its filtration in kidney renal tubules increase causing calcification leads to nephrogenic diabetes inspiidus)
- Renal diseases such as glomerulonephritis and cystitis
- urinary tract infection Although it more commonly causes frequent passage of small volumes of urine rather than a large volume

> **Note:** *High volume with High specific gravity may indicate D.mellitus and High volume with low specific gravity may indicate D.Insipidus while*

Oliguria

Oliguria are the decreased production of urine. It is defined as urine output that is less than 400 ml/day in adults

What are the causes of oliguria?

Physiological causes

- Reduced fluid intake
- Exercise that cause sweating

Pathological causes

Pre-renal causes (in response to hypoperfusion of the kidney)

- Dehydration caused by prolonged vomiting, diarrhea, or burns
- Cardiac insufficiency

Renal causes (due to kidney damage)

- Nephritic syndrome
- post-streptococcal glomerulonephritis
- Acute tubular necrosis

Post-renal causes (as a consequence of obstruction of the urine flow)

- Enlarged prostate
- Tumor compression urinary outflow

Note: *oliguria usually cause increased specific gravity except in acute tubular necrosis because the tubules do not concentrate the glomerulr filtrate*

Anuria

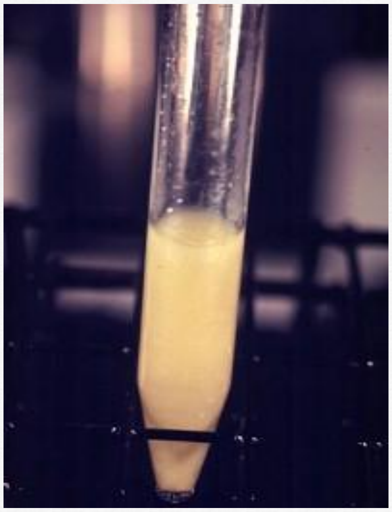
Also sometimes called anuresis and it means non passage of urine. But it is practically defined as passage of less than 50 ml of urine in a day.

What are the causes of anuria?

- Bilateral complete urinary tract obstruction (an enlarged prostate gland is a common cause of obstructive anuria)
- It may occur with end stage renal disease.

Urine Aspect

Overview:



The turbidity of the urine sample is gauged subjectively and reported. Normally, fresh urine is clear to very slightly cloudy.

Note: *Pathologic urines are often turbid or cloudy; however, normal urine can also appear cloudy.*

Procedure:

Observe the clarity of a fresh urine sample by visually examining a well-mixed specimen in front of a light source.

Common terms used to report appearance include the following:

Clear, hazy, slightly cloudy, cloudy, turbid, and milky.

Note: *the degree of turbidity should correspond to the amount of material observed under the microscope.*

Interpretation:

Materials which impart turbidity to the urine are: Bacteria ,Epithelial cells , Erythrocytes (urine pink or red) ,Leukocytes, Mucus, Crystals, Amorphous, sperms

Note: *vaginal discharges mixed with urine or fecal contamination is common causes of turbidity.*

Urine Color

Overview:

Normal urine color ranges from pale yellow to deep amber
The yellow color of urine is caused by the presence of the pigment urochrome, a product of hemoglobin metabolism that under normal conditions is produced at a constant rate.
Urine color varies, depending on how much water you drink.

Fluids dilute the yellow pigments in urine, so the more you drink, the clearer your urine looks. When you drink less, the color becomes more concentrated

Note: *Normal urine color darkens on standing because of the oxidation of urobilinogen to urobilin. This decomposition process starts about 30 minutes after voiding.*

Procedure:

Observe and record the color of freshly voided urine.

Interpretation:

Very pale yellow or colorless urine

Diabetes Militus , Diabetes Insipidus , Alcohol ingestion (inhibit ADH release) ,caffeine ingestion (increase GFR by dilating afferent arterioles) , Diuretic therapy

Deep yellow urine

Dehydration or drinking too few fluids can concentrate urochrome, making urine much deeper in color.
Also found in concentrated urine caused by fever, sweating, reduced fluid intake, or first morning specimen
A number of drugs can darken urine, including the antimalaria drugs chloroquine and primaquine; the antibiotic metronidazole; and nitrofurantoin

Red or pink urine

The presence of red blood cells is the main reason that urine turns cloudy red (Smoky urine)

Greenish-yellow urine

May indicate bilirubin in the urine (give greenish foam when shaken)

Urine Specific gravity

Overview:

Specific gravity is defined as the ratio of the density of a given solid or liquid substance to the density of water at a specific temperature and pressure. Substances with a specific gravity greater than one are denser than water, and so will sink in it, and those with a specific gravity of less than one are less dense than water, and so will float in it.
Urine specific gravity measures urine density, or the ability of the kidney to concentrate or dilute the urine.
The USG is influenced by the number of molecules in urine, as well as their molecular weight and size.

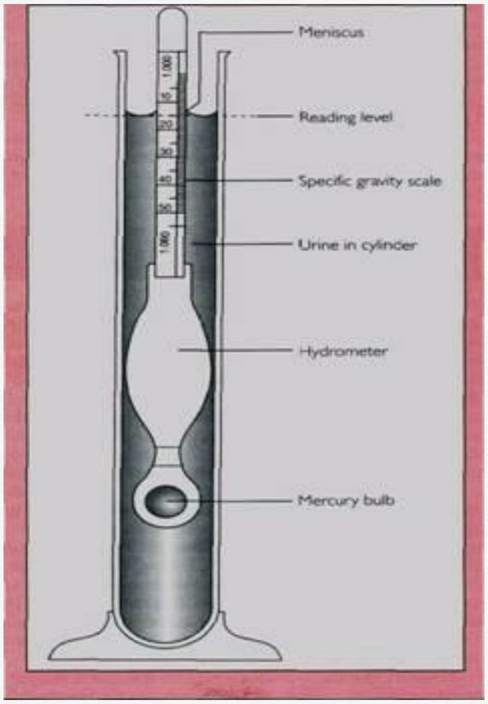
Note: *urine specific gravity is directly proportional to urine osmolality which measures solute concentration*

Procedure:

The urinometer (hydrometer)

Principle

It is most widely known but least accurate method.
It consists of a bulb-shaped instrument that contains a scale calibrated in SG readings.
Urine (10 to 20 ml) is transferred into a small cylinder, and the urinometer is floated in the urine.
The SG is read off the urinometer at the meniscus level of the urine.



Interference

Temperature of urine specimens affects SG; cold specimens produce falsely high values using the hydrometer.

For correcting the urinometer reading we can use the following formula:

Sp.Gr At 25C = (Room Temp - 25) / 3 + Reading

Note: *if urine sample is little , dilute urine then measure* specific gravity *then multiply the last two digits by the dilution factor*

Normal

The range of urine SG depends on the state of hydration and usually between 1.015and 1.025

Interpretation:

Reduced specific gravity (Hyposthenuria) (1.001 -1.010)

- Diabetes insipidus
- excess fluid intake
- Pyelonephritis
- Glomerulonephritis
- Treatment with diuretics

Raised specific gravity (Hypersthenuria) (1.025 -1.035)

- Diabetes mellitus
- Adrenal insufficiency
- Congestive cardiac failure (related to decreased blood flow to the kidneys)
- Excessive sweating
- Excessive water loss (dehydration, fever, vomiting, diarrhea)
- Toxemia of pregnancy
- Cystitis - products of inflammatory reaction are added to the urine

Urine chemical examination

Procedure

- Dip the test areas of the strip in the urine specimen (fresh, well mixed and un-centrifuged).
- Remove excess of the urine by tapping the edge of the strip against the container.
- Compare the test areas closely with corresponding color charts on the bottle at the times specified.

  Note: *The strip should be compared with the corresponding color charts in reasonably good light.*

Interfering Factors

1. If the dipstick is kept in the urine sample too long, the impregnated chemicals in the strip might be dissolved and could produce inaccurate readings and values.
2. If the reagent chemicals on the impregnated pad become mixed, the readings will be inaccurate.
3. Precise timing is essential. If the test is not timed correctly, color changes may produce invalid or false results.
4. Discoloration of the urine by bilirubin, blood or other constituents.
5. If the reagents absorb moisture from the air before they are used, they will not produce accurate results.

الاحتياطيات الواجب مراعاتها عند الاستخدام:

- حفظ الـشرائط من الرطوبة والحرارة الزائدتين فى وعاء محكم الغلق
- حفظ الـشرائط في منطقة جافة وباردة ولكن ليس في الثلاجة.
- عند الإست خدام يجب أن تكون الـشرائط في نفس درجة حرارة الغرفة.
- يجب الحرص على أن لا نلمس مناطق الـشرائط بالأصابع.
- يجب التأكد من أن الـشرائط صالحة للاستخدام من ناحية الصلاحية.
- لا تستخدم الـشرائط في وجود أبخرة حمضيه أو قاعدية لأن ذلك يؤثر على نتيجة التفاعل الكيميائي مهما كانت هذه الأبخرة بسيطة.
- يجب التأكد من أن الـشريط ثم تغميسه كاملا في البول
- وتجنب بقاء الشريط فترة طويلة ملامسا للبول
- يجب القراءة في وجود ضوء مناسب.
- لاحظ أن تغير لون الكاشف يدل علي أنها فقدت حساسيتها
- لابد أن يتم التخلص من الشرائط المستعملة أولا بأول حتى لا يعاد استخدامها بالخطأ مرة أخرى

Urine Reaction

Overview:

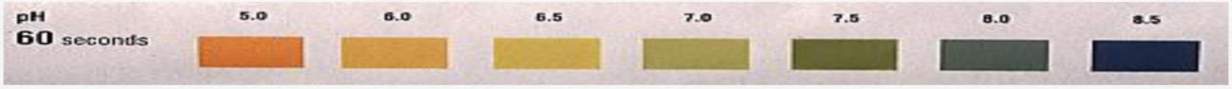
pH is a measure of the acidity or basicity of a solution. It is defined as the cologarithm of the activity of dissolved hydrogen ions (H^+).
The pH is an indicator of the renal tubules ability to maintain normal hydrogen ion concentration in the plasma and extracellular fluid.
The kidneys maintain normal acid-base balance primarily through re-absorption of sodium and tubular secretion of hydrogen and ammonium ions.

Importance

- Precipitation of urine crystals in supersaturated urine is highly dependent on urine pH (e.g. struvite will precipitate in alkaline not acidic urine).
- Knowledge of the urine pH is important in interpreting urine sediment findings for example: erythrocytes, leukocytes, and casts tend to disintegrate in alkaline urine (pH > 8.0
- some medications are more effective in acidic or alkaline environments. For example, streptomycin, neomycin, and kanamycin are more effective in treating urinary tract infections when the urine is alkaline.
- Control of urinary pH is important in the management of several diseases, including :
  - The urine should be kept acid during treatment of UTI or persistent bacteriuria
  - The urine should be kept acid during management of urinary calculi that develop in alkaline urine.

Procedure:

Using chemical strip testing



The test is based on the double indicator (methyl red/bromthymol blue) principle that gives a broad range of colors covering the entire urinary pH range. Colors range from orange through yellow and green to blue.

Interfering Factors

- With prolonged standing, the pH of a urine specimen becomes alkaline because bacteria split urea and produce ammonia.
- "Runover" between the pH testing area and the highly acidic protein area on the dipsticks may cause alkaline urine to give an acidic reading.

Normal:

The pH of normal urine can vary widely, from 4.6 to 8.0 (The average pH value is about 6.0 (acid)

> **Note:** *The pH of urine never reaches 9, either in normal or abnormal conditions. Therefore, a pH finding of 9 indicates that a fresh specimen should be obtained to ensure the validity of the urine analysis.*

Interpretation:

Acidic urine (pH <7.0) occurs in

- diabetic ketosis
- prolonged diarrhea
- starvation
- UTIs caused by Escherichia coli
- gout

- Highly concentrated urine, such as that formed in hot, dry environments, is strongly acidic and may produce irritation.
- During sleep, decreased pulmonary ventilation causes respiratory acidosis; as a result, urine becomes highly acid.

Alkaline urine (pH >7.0) occurs in

- **Post-prandial alkaline tide**
  Alkaline urine after meals is a normal response to the secretions of hydrochloric acid in gastric juice (usually starts within 15-20 minutes after eating food).
- UTIs caused by urea-splitting bacteria (Proteus and Pseudomonas) (Persistent alkaline urine suggests urinary tract infection)
- prolonged vomiting

Urine Albumin

Overview

In a healthy renal and urinary tract system, the urine contains no protein or only traces amounts. These consist of albumin (one-third of normal urine protein is albumin) and globulins from the plasma.
Normally, more than 99% of the filtered protein is reabsorbed by pinocytosis in the proximal convoluted tubule

- **Albuminuria**

Because albumin is filtered more readily than the globulins, it is usually abundant in pathologic conditions; therefore, the term albuminuria is often used synonymously with proteinuria.

> **Note:** *If more than a trace of protein is found persistently in the urine, a quantitative 24-hour evaluation of protein excretion is necessary.*

Procedure:

1- Dipstick test:

Principle

This test is based on the "protein error of indicator dyes(tetrabromphenol blue)". Basically, the test is dependent on the ability of amino groups in proteins to bind to and alter the color of acid-base indicators, even though the pH is unchanged.

> **Note:** *The reaction is extremely sensitive to albumin (as it contains the most amino groups), but is much less sensitive to globulins and hemoglobin and it is insensitive to Bence-Jones proteins.*

> **Note:** *Very dilute urine may give a falsely low protein value.*

> **Note:** *If positive urine strips test must be followed by other confirmatory tests*

False positive reactions

- False positives occur rarely in highly alkaline urine samples.
- If urine containing residues of detergents,
- Urine of high specific gravity may cause higher than normal readings

2- Coagulation test (heating)

A tube filled with urine is heated using flame and take care to prevent effervescence

Results

If no ppt. is formed so no protein in this urine sample

If white ppt is formed add few drops from GAA

- If ppt. is removed so this is amorphous phosphate ppt.
- If ppt. is remain as it is so it will be albumin (due to protein denaturation by heat)

Interpretation:

Physiological proteinuria

- Transient proteinuria (functional proteinuria)
- Intermittent proteinuria (orthostatic proteinuria)

Pathological proteinuria

- prerenal proteinuria (overflow proteinuria)
- Renal proteinuria
  - Glomerular proteinuria (albuminuria)
  - Tubular proteinuria
- Post-renal proteinuria

Urine Sugar

Overview:

Glycosuria or ***glucosuria*** is an abnormal condition of osmotic diuresis due to excretion of glucose by the kidneys into the urine.

The most common cause of glycosuria is untreated diabetes mellitus which raises plasma glucose levels far above normal, and beyond a certain threshold, the excess glucose is excreted by the kidneys, taking water with it and producing diuresis.

The threshold varies somewhat from one individual to another, with values around (160 - 180 mg/dl)

Procedure:

Chemical strip testing

Principle

This test is based on a double sequential enzyme reaction. One enzyme, glucose oxidase, catalyzes the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose. A second enzyme, peroxidase, catalyzes the reaction of hydrogen peroxide with a potassium iodide chromogen to oxidize the chromogen to colors ranging from green to brown.

Note: *These tests are specific for glucose only.*

False positive reactions

- The presence of hydrogen peroxide, bacterial peroxidases (e.g. cystitis), will produce false positive reactions.
- Drugs: Nalidixic acid, cephalosporins, Chloramphenicol, Isoniazid and penicillin
- Stress, excitement, testing after a heavy meal, and testing soon after the administration of intravenous glucose may all cause false-positive results, most frequently trace reactions.

False negative reactions

- High concentrations of ascorbic acid (>25 mg/dl) inhibit the reaction.
- Drugs: salicylates, tetracyclines.
- False-negative results may occur if urine is left to sit at room temperature for an extended period.
- High specific gravity depresses color development.

Interpretation:

Glucosuria is the excretion of glucose in the urine which may occurs:

when plasma glucose concentration exceeds its renal tubular absorptive capacity [renal glucose threshold: (l80 mg/d)].

Hyperglycemia without glucosuria occurs when the renal threshold for glucose is raised.

There may be glucosuria without hyperglycemia as in low renal threshold for glucose or renal tubular defects.

Urine ketone

Overview:

Ketone bodies are three water-soluble compounds that are produced as by-products when fatty acids are broken down for energy in the liver and kidney. They are used as a source of energy in the heart and brain. In the brain, they are a vital source of energy during fasting. The three ketone bodies are acetone, acetoacetic acid, and beta-hydroxybutyric acid.

ketonemia (Ketosis): is a state characterised by elevated levels of ketone bodies in the blood, occurring when the liver converts fat into fatty acids and ketone bodies (which can be used by all of the body for energy as an alternative to glucose).

Ketonuria: is a medical condition in which ketone bodies are present in the urine. Ketones will be present in the urine when the ketones in the blood go above a certain level.

What is the indication for urine ketone test?

- **Screening for ketonuria in pregnant women**

  During pregnancy, the early detection of ketones is essential because ketoacidosis is a prominent factor that contributes to intrauterine death.

- **Screening for ketonuria in persons with diabetes**

  1. Testing for ketones is indicated in any patient showing elevated urine and blood sugars (blood sugar levels of 300 mg/dl or higher)
  2. When treatment is being switched from insulin to oral hypoglycemic agents, the development of ketonuria within 24 hours after withdrawal of insulin usually indicates a poor response to the oral hypoglycemic agents.
  3. Ketone testing is done to differentiate between diabetic coma positive ketones and insulin shock negative ketones.

Procedure:

Chemical strip testing

Principle

This test is based on the development of colors ranging from buff-pink, for a negative reading, to purple when acetoacetic acid reacts with nitroprusside.

Urine testing only detects acetoacetic acid, not the other ketones, acetone or beta-hydroxybuteric acid.

Interfering Factors

- False-positive results may caused by drugs such as Penicillamine and depakene
- False-negative results occur if urine stands too long, owing to loss of ketones into the air.

Interpretation:

Metabolic disease

- Diabetes mellitus (diabetic acidosis)
- Hyperthyroidism

Dietary conditions

- Starvation, fasting
- High-fat diets
- Prolonged vomiting, diarrhea (cause dehydration)

Increased metabolic states caused by:

- prolonged Fever
- Pregnancy or lactation
- strenuous exercise
- severe stress
- during acute illness (Approximately 15% of hospitalized patients have ketones in their urine even though they do not have diabetes)
- Post-surgical condition (Ketonuria occurs after anesthesia (ether or chloroform))

Urine Nitrite

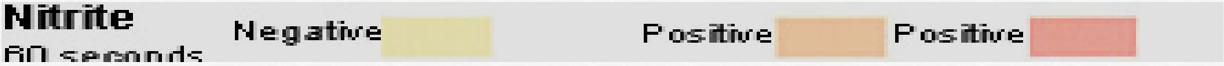
Overview:

Whereas ***NITRATES*** are normal in urine (mainly coming from food additives and from food protein,), the presence of ***NITRITES*** is not normal.

Bacteria that cause a urinary tract infection (some gram-negative bacteria) produce an enzyme (*nitrate reductase*) that converts urinary nitrates to nitrites. The presence of nitrites in urine indicates therefore a UTI (urinary tract infection).

Procedure:

Urine stripe



Principle

At the acid pH of the reagent area, nitrite in the urine reacts with para-arsanilic acid to form a diazonium compound. This diazonium compound in turn couples with 1,2,3,4-tetrahydrobenzo(h)quinoline-3-ol to produce a pink color

The sensitivity of the urine dipstick test for nitrites has been found to be low (45 %- 60% in most situations) with higher levels of specificity (85 %- 98%).

Note: *The color intensity is not significant, and is not proportional to the number of bacteria present in the urine sample.*

Interfering Factors

False negative results

- High doses of vitamin C may cause false-negative test for nitrite on dipstick.
- urine has not incubated in patient's bladder for ≥4 hours cause false-negative test
- False-negative reaction may be caused by some important bacteria that do not reduce dye (Gram-positive as staphylococci, streptococci don't contain the enzyme reductase)
- The bacterial enzymes that reduce nitrate to nitrite can convert nitrite to nitrogen so give false –ve result
- Sensitivity decreases if sufficient dietary nitrate are not be present for the nitrate-to-nitrite reaction to occur.

False positive results

- bilirubin can produce false-positive results.
- False-positive results can be obtained if the urine sits too long at room temperature, allowing contaminant bacteria to multiply.

Interpretation:

A positive nitrate test is a reliable indicator of significant bacteriuria (especially E Coli)

Urine Bilirubin

Overview:

Bilirubin is the yellow breakdown product of normal heme catabolism.

Procedure:

Chemical strip testing

Principle

This test is based on the coupling of bilirubin with diazotized dichloroanaline in a strongly acid medium. The color ranges through various shades of tan. The test is sensitive to 0.2-0.4 mg/dL of conjugated bilirubin.

> **Note:** *Examine the urine within 1 hour of collection because urine bilirubin is unstable, especially when exposed to light.*

> **Note:** *If the urine is yellow-green to brown, shake the urine. If yellow-green foam develops, bilirubin is probably present.*

Interfering Factors

High concentrations of ascorbic acid cause decreased sensitivity.

Aged urine samples

Iodine test

add drops of iodine carefully on the tube wall if green colored ring developed gradually between iodine and urine this indicate that bilirubin is probably present.

Normal:

Normally, a tiny amount of bilirubin is excreted in the urine, accounting for the light yellow color.

> **Note:** *urine bilirubin is reported a follow negative, +, ++, +++*

Interpretation:

Jaundice, also known as ***icterus*** is a yellowish discoloration of the skin, the conjunctival membranes over the sclerae (whites of the eyes), and other mucous membranes caused by hyperbilirubinemia (increased levels of bilirubin in the blood).

> **Note:** *Typically, the concentration of bilirubin in the plasma must exceed 1.5 mg/dl, for the coloration to be easily visible.*

Causes of jaundice

Jaundice is classified into three categories, depending on which part of the physiological mechanism the pathology affects.

The three categories are:

- **Pre-hepatic:** The pathology is occurring prior the liver
- **Hepatic:** The pathology is located within the liver
- **Post-Hepatic:** The pathology is located after the conjugation of bilirubin in the liver

Urine Urobilinogen

Overview:

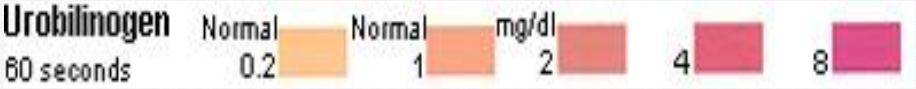
Urobilinogen is a colourless product of bilirubin reduction. It is formed in the intestines by bacterial action.

Some urobilinogen is reabsorbed, taken up into the circulation and excreted by the kidney. This constitutes the normal "***intrahepatic urobilinogen cycle***". So normally urine have trace amounts of urobilinogen.

Procedure:

Chemical strip testing

Principle



This test is based on the modified Ehrlich reaction, in which para-diethylaminobenzaldehyde in conjunction with a color enhancer reacts with urobilinogen in a strongly acid medium to produce a pink-red color.

Interfering Factors

- A false positive urobilinogen reaction may occur with the dipstick method when substances known to react with Ehrlich's reagent such as sulfonamides are present in the urine.
- False Negative result may be due to the instability of urobilinogen, a false negative result may occur using a dipstick method if the urine specimen has remained at room temperature for an extended period of time in the light.

Interpretation:

Increased urobilinogen

Urinary urobilinogen is increased by any condition that causes an increase in the production of bilirubin such as: Pre-hepatic Jaundice and Hepatic Jaundice.

Low or absence of urobilinogen

May be caused by

- Post-hepatic Jaundice
- Impaired intestinal absorption (i.e., diarrhea)
- During broad-spectrum antibiotic therapy, suppression of normal gut flora may prevent the breakdown of bilirubin to urobilinogen; therefore, urine levels will be decreased or absent.

Urine microscopic examination

Introduction:

Microscopic examination of urine sediment is of great clinical importance and should never be omitted.
The sediment should be examined for: Type, and Amount
Examination of the sediment should always be made shortly after collection so that:

a. Degeneration and lysis of cellular elements will not occur
b. Bacteria will not proliferate

Procedure:

A. Centrifugation

- Shake the urine sample to make the sample homogenous.
- Put (9-11) ml of urine sample into test tube.
- The recommended parameter for the urine centrifugation is 5 minutes at 2000 RPM.

Note: *Specimens must not be overspun because with compacted pellets, resuspension is more difficult, leucocytes and some other elements form clumps and this will give unevenly distributed slides.*

B. Re-suspension

- The re-suspension procedure has to provide the better homogeneous distribution possible.
- An inadequate re-suspension can be the cause of an uneven distribution although, in the presence of mucus, to which elements may adhere, can cause a significant variation in the different field counts.

C. Examination

- Place a drop of unstained suspension in a glass slide
- Place the glass slide on the microscope stage. Examine several fields at 10X and 40X magnification
- Classify and count casts within LPF (Low Power Fields).
- Switch to 40X magnification and examine for other elements, i.e., WBCs, RBCs, Epithelial cells, yeast, bacteria, Sperm cells, mucous filaments and crystals.

Urine Pus cells

Overview:

Leucocytes usually enter tubular lumen through and between tubular epithelial cells
An increase in urinary WBCs is called ***pyuria*** and indicates the presence of an infection or inflammation in the genitourinary system.
Microscopic examination and chemical testing are used to determine the presence of leukocytes in the urine.

Procedure:

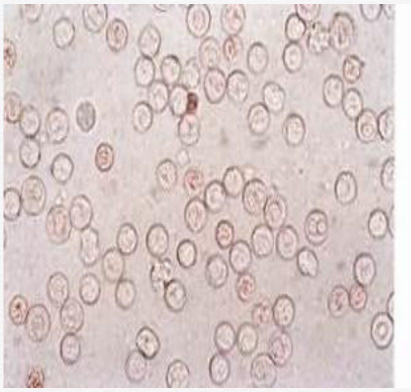
WBC is reported semi-quantitatively as number seen per high power field (HPF) and reported as follow; <5, 5-20, 20-100, or >100 (Over 100).

Normal:

WBC up to 5/HPF are commonly accepted as normal.

Appearance:

These white blood cells in urine have lobed nuclei and retractile cytoplasmic granules.



White Blood Cells (WBC) in unstained urine sediments typically appear as round, granular cells which are 1.5-2.0 times the diameter of RBCs.

> **Note:** *WBC in urine is most commonly neutrophils but nuclei tend to become round as neutrophils age in urine.*

> **Note:** *Like erythrocytes, WBC may lyse in very dilute or highly alkaline urine; WBC cytoplasmic granules released into the urine often resemble cocci bacteria.*

> **Note:** *to differentiate between neutrophils and RBCs cells a small drop of GAA is added which enhance the nuclear details and lyse the red blood cells.*

Interpretation:

Greater numbers of WBCs (***pyuria***) generally indicate the presence of an inflammatory process somewhere along the course of the urinary tract.

Positive results are clinically significant and indicate:

- Acute pyelonephritis
- Cystitis
- prostatitis
- urethritis

Other causes of inflammation must be considered, such as:

- Kidney stone
- Urinary tract neoplasm, including renal cancer and bladder cancer.
- Acute Glomerulonephritis (non bacterial)

> **Note:** *In bladder infections, WBCs tend to be associated with bacteria, epithelial cells, and relatively few RBCs.*

> **Note:** *WBC clumps suggest renal origin of WBCs and should be reported when present.*

> **Note:** *Pus count greater than 30\HPF suggest acute infection and urine culture is recommended*

Sterile pyuria

Sterile pyuria is urine which contains white blood cells (>10 white cells/mm3) while appearing sterile by standard culturing techniques.

Causes

- A recently (within last 2 weeks) treated urinary tract infection (UTI)
- UTI with 'fastidious' organism (an organism that grows only in specially fortified artificial culture media under specific culture conditions), e.g. Neisseria gonorrhoeae and Renal tuberculosis.
- Urinary tract stones.
- Also sterile pyuria is listed as a side effect from some medications such as paracetamol (acetaminophen)

Urine RBCs

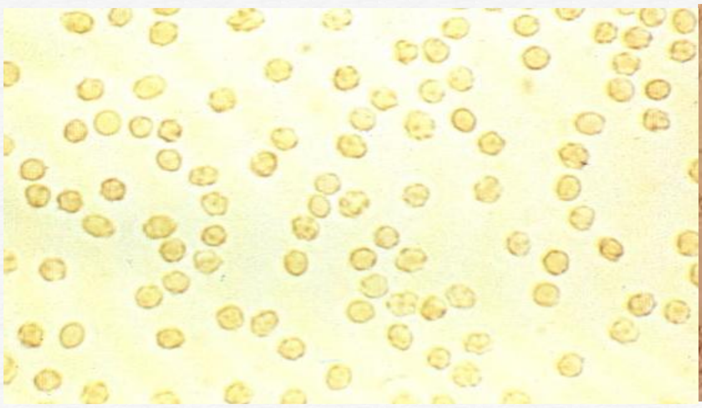
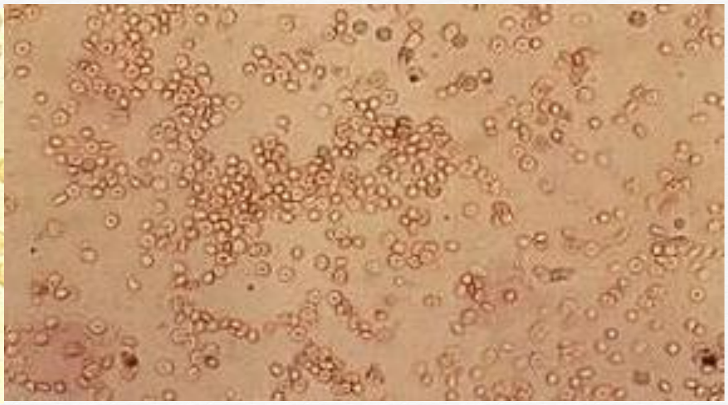
Overview:
Theoretically, no red cells should be found, but some find their way into the urine even in very healthy individuals.
Increased red cells in urine above normal level is termed ***hematuria***

Procedure:
RBCs is reported semi-quantitatively as number seen per high power field (HPF) and reported as follow; <5, 5-20, 20-100, or >100.

Normal:
RBC up to 5/HPF are commonly accepted as normal.

Appearance:
The appearance of red blood cells (RBC) in urine depends largely on the concentration of the specimen and the length of time the red cells have been exposed.

- Fresh red cells tend to have a red or yellow color and appear as retractile disks
- Prolonged exposure results in a pale or colorless appearance as hemoglobin may be lost from the cells and the RBC's begin to have a crenated appearance specially in concentrated urine (hypertonic urine).

Note: *erythrocytes may lyse in very dilute or highly alkaline urine; Lysed red cells appear as very faint "ghosts", or may be virtually invisible. (Red cell ghosts may simulate yeast)*

Note: *RBC's in urine may be confused with oil droplets or yeast cells but remember that: Oil droblets exhipt great variation in size and highly refrectile and yeast cells usually show budding , But if there are drought about identification a few drops of GAA is added to the slide causing RBCs is lyses by acidification*

Note: *The presence of dysmorphic RBC's (distorted cell) in urine suggests a glomerular disease such as a glomerulonephritis. Dysmorphic RBC's have odd shapes as a consequence of being distorted via passage through the abnormal glomerular structure.*

Note: *the presence of increased number of RBCs with casts and proteiuria suggests bleeding from renal origin*

Note: *growth hematuria sugesset bledding origin in urethra or bladder neck*

Interpretation:

hematuria is the presence increased amount of red blood cells (erythrocytes) in the urine.

What are the types of hematuria?

Microscopic hematuria

Small amounts of blood, can be seen only on urinalysis or light microscopy), In microscopic hematuria, the urine appears normal.

Macroscopic hematuria (or "frank" or "gross") hematuria

Gross hematuria is suspected because of the presence of red or brown urine.

Note: *Typically, microscopic hematuria indicates damage to the upper urinary tract (kidneys), while visible blood indicates damage to the lower tract (ureters, bladder, or urethra). But this is not always the case.*

What are the Causes of hematuria?

Renal causes

- **Post-streptococcal glomerulonephritis):** It is the commonest cause of haematuria in children above 3 years. History of preceding streptococcal pharyngitis is usually obtained.

  Note: *The prognosis of post-streptococcai glomerulonephritia is excellent and complete recovery within few weeks occur but microscopic haematuria may remain for several months.*

- **Urolithiasis (Urinary calculi):** recurrent attacks of haematuria (intermittent hematuria) with abdominal pain or dysuria should suggest the possibility of urinary stones.

Non-renal Causes

- Strenuous exercise
- Urinary Schistosomiasis
- Cystitis: diagnosis is confirmed by the presence of pyuria and bacteriuria.
- Haematological disorders as thrombocytopenia and hemophilia

Urine Castes

Overview:

Urinary casts are cylindrical aggregations of particulate matter that form in the distal nephron, dislodge, and eventually pass into the urine.

Tamm-Horsfall protein

Uromodulin, also known as UMOD, Tamm-Horsfall protein (THP), or Tamm-Horsfall mucoprotein, is the most abundant protein in normal urine. normal daily excreted quantity ranges from 25 to 50 mg.

Function

Uromodulin may act as a constitutive inhibitor of calcium crystallization in renal fluids, and t may provide defense against urinary tract infections.

Lindner's mechanism of cast formation

The cast matrix is formed of uromucoprotein fibrils and is builded by a mechanism described by Lindner. The mechanism has four steps:

Initiation

The first Tamm-Horsfall protein fibrils are fixed to the distal tubular walls, forming a porous sponge like lattice. Since the initial cast has large pores, urine and small debris pass through.

This stage could correspond to the early hyaline cast.

Growth

As time goes, more and more elementary fibrils, and maybe other proteins, are added to the initial structure, making the pore smaller. The declining pores size reduces the urine flow through the structure.

Maturation

After complete obstruction of urine flow, the cast matrix is modified by the tubular activity. The maturation period depends on the nephron activity, especially at the blockade region.

Proteins, originating from the surrounding tubular cells, are added to the structure.

With renal tubular injury, epithelial cells slough into the lumen of the renal tubules and precipitated on the mucoprotein matrix.

With time, the epithelial cells degenerate and can no longer be recognized as cells within the hyaline matrix, thus forming coarsely granular, then finely granular, casts. Waxy casts are the final step in the formation of casts and usually indicate chronic tubular disease.

Evacuation

Because of the surrounding cells activity and the hydrostatic pressure, a time comes where the cast loses its adherence to the tubular wall. The casts are then evacuated by the urinary flow.

Factors known to be promoters of cast formation are:

- Increased amount of certain proteins (Bence-Jones, myoglobin, Albumin, hemoglobin...)
- Urinary stasis
- Cellular debris
- Low glomerular filtration rate
- Acidic pH

Normal:

They are absent or very few in urine samples

> **Note:** *Casts are quantified for reporting as the number seen per low power field (10x objective) and classified as to type (e.g., hyaline casts).*

Appearance:

The appearance of a cast observed in a urine sediment depends largely upon the length of time it remained in situ in the tubules prior to being shed into the urine, as well as where it forms in the tubules.

Types of casts

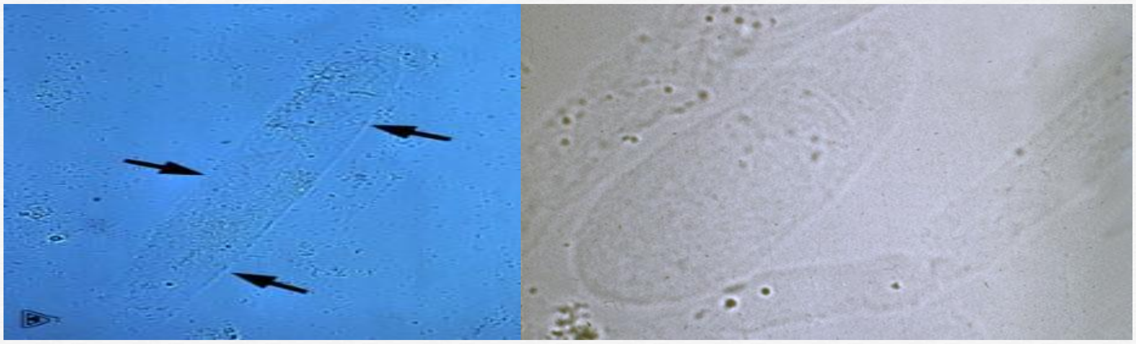
The various types of casts that can be found in urine sediment may be classified as follows.

Hyaline casts

Appearance:

The most common type of cast, hyaline casts are solidified Tamm-Horsfall mucoprotein secreted from the tubular epithelial cells of individual nephrons.

Hyaline casts are cylindrical and clear, with a low refractive index, so that they can easily be missed under bright field microscopy or on an aged sample where dissolution has occurred.



Interpretation:

When present in low numbers (0-1/LPF) in concentrated urine of normal patients, hyaline casts are not always indicative of clinically significant renal disease and may be association with:

- Fever (dehydration)
- Emotional stress
- Strenuous exercise
- Heat exposure

Granular casts

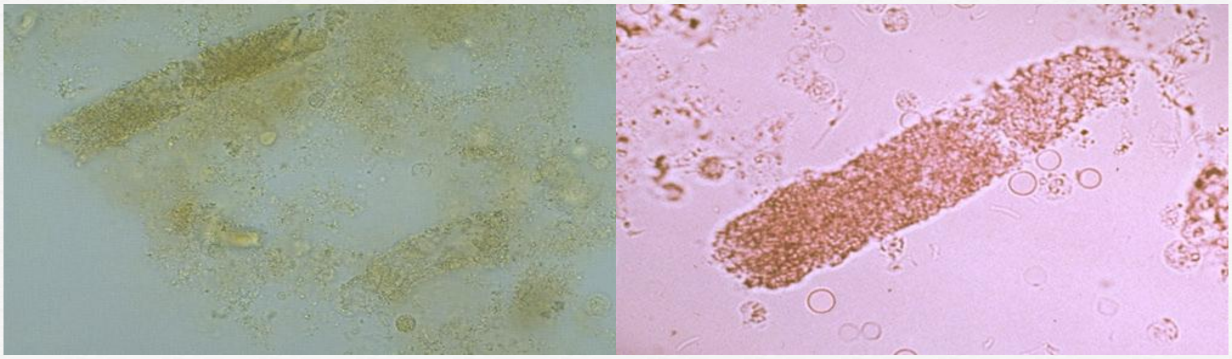
Appearance:

It can result either from the breakdown of cellular casts (remain in the nephron for some time before they are flushed into the urine), or the inclusion of aggregates of plasma proteins (eg, albumin) or immunoglobulin light chains.

Depending on the size of inclusions, they can be classified as fine or coarse, though the distinction has no diagnostic significance.

Appearance is generally more cigar-shaped and of a higher refractive index than hyaline casts.

> **Note:** *amorphous materials and crystals may precipitate on mucus threades and give the appearance of true granular casts*



Interpretation:

Its appearance is most often indicative of chronic renal disease such as:

- Glomerulonephritis
- Pyelonephritis

> **Note:** *An absence of casts does not rule out renal disease. Casts may be absent or very few in cases of chronic, progressive nephritis. Even in cases of acute renal disease, casts can be few or absent in a single sample since they tend be shed intermittently. Furthermore, casts are unstable in urine and are prone to dissolution with time, especially in dilute and/or alkaline urine.*

Urine Crystals

Overview:

A variety of crystals may appear in the urine. They can be identified by their specific appearance and solubility characteristics. Crystals in the urine may present no symptoms, or they may be associated with the formation of urinary tract calculi and give rise to clinical manifestations associated with partial or complete obstruction of urine flow.

Why urine crystals are formed?

When the amount of solutes in urine increase (due to dehydration, dietary intake, or medications) urine super-saturation occurs and crystals will be formed either while the urine in the body or after the urine is voided

Factors influence the types and numbers of urinary crystals

In vivo factors include:

- the concentration and solubility of crystallogenic substances contained in the specimen,
- the urine pH, and
- The excretion of diagnostic and therapeutic agents.

In vitro factors include:

- temperature (solubility decreases with temperature),
- evaporation (increases solute concentration), and
- Urine pH (changes with standing and bacterial overgrowth).

Procedure:

- Collect a random urine specimen.

  Note: *Crystal identification should be done on freshly voided specimens.*

- Examine the urinary sediment microscopically under high power.
- The pH of the urine is an important aid to identification of crystals and must be noted.

Interfering Factors

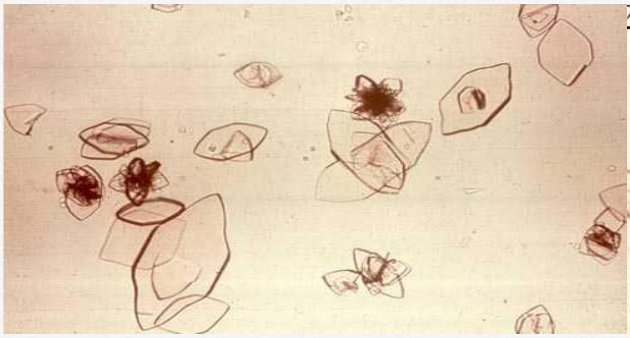
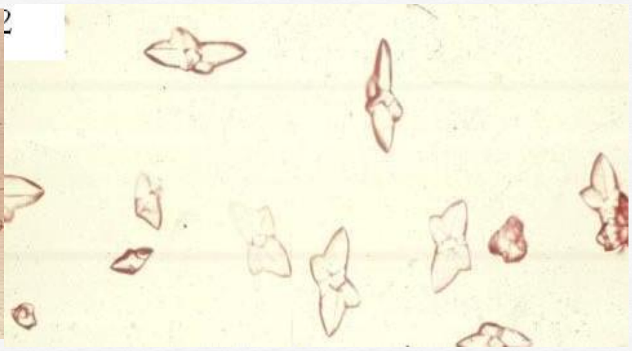
- Refrigerated urine will precipitate out many crystals because the solubility properties of the compound are altered.
- Urine left standing at room temperature will also cause precipitation of crystals or the dissolving of the crystals.

Appearance:

Uric Acid crystals

Pure uric acid crystals are colorless but the sediment in urine is impure so these crystals usually appear pigmented. Uric acid crystals may appear as yellow to brown rhombic or hexagonal plates, needles or rosettes.

It may be found in gout, kidney stones, chronic nephritis

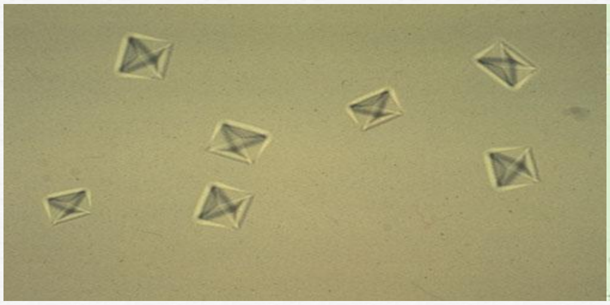



Calcium Oxalate crystals (Dihydrate)

Calcium oxalate dihydrate crystals typically are seen as colorless squares whose corners are connected by intersecting lines (resembling an envelope).
The crystals vary in size from quite large to very small.

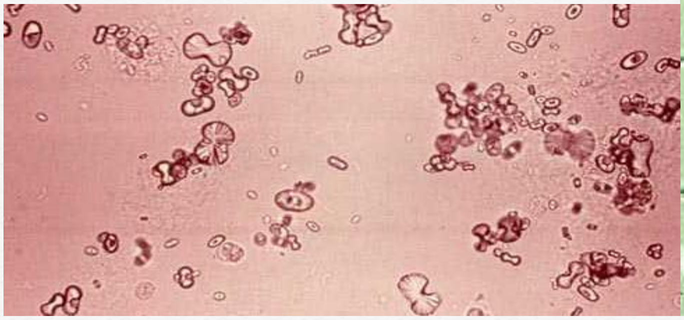
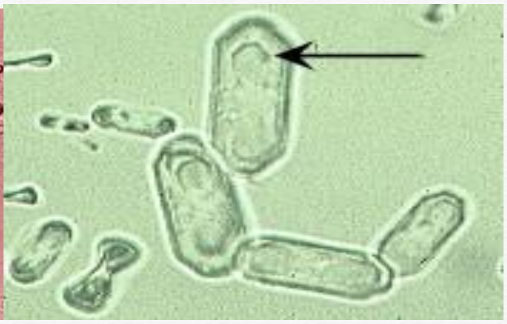
> **Note:** *In some cases, large numbers of tiny oxalates may appear as amorphous unless examined at high magnification.*

Hypercalciuria (may be caused by hyper-parathyroidism) causes stones in more than half of cases (present in 50% of men and 75% of women with Ca calculi) In this condition, too much calcium is absorbed from food and excreted into the urine, where it may form calcium oxalate stones.



Calcium Oxalate crystals (monohydrate)

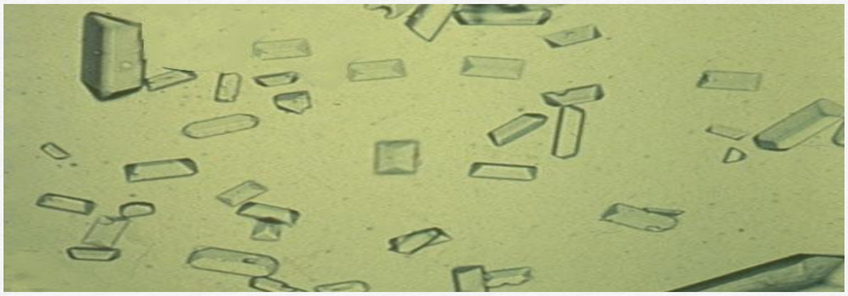
Calcium oxalate monohydrate crystals vary in size and may have a spindle, oval, or dumbbell shape. Found normally in urine after ingestion of vit C, tomato, spinch , garlic , orange

Triple phosphate crystals

Struvite is also occasionally referred to as “Triple Phosphate” due to an old erroneous belief that the phosphate ion was bonded to 3 positive ions instead of just magnesium and ammonium.
Struvite crystals appears as colorless, 3-dimensional, prism-like crystals ("coffin lids").
Though they can be found in urine of any pH, their formation is favored in neutral to alkaline urine.
They are found in urine in cases of chronic cystitis

> **Note:** *Urinary tract infection with urease producing bacteria can promote struvite crystalluria by raising urine pH and increasing free ammonia.*



Urine Amorphous

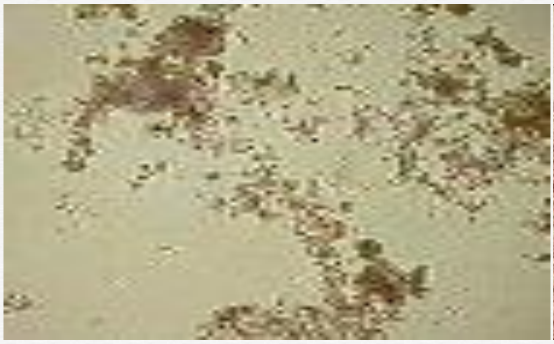
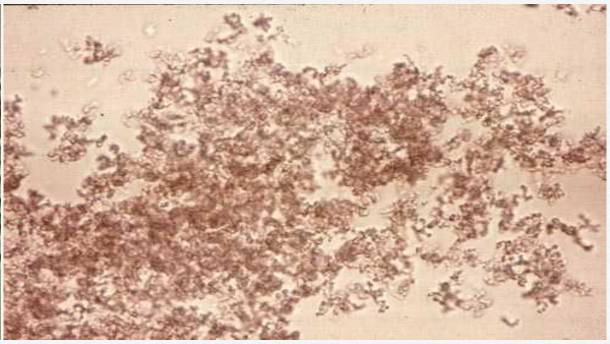
Overview:

Amorphous crystals appear as aggregates of finely granular material without any defining shape. Generally, no specific clinical interpretation can be made for the presence of amorphous crystals.

Appearance:

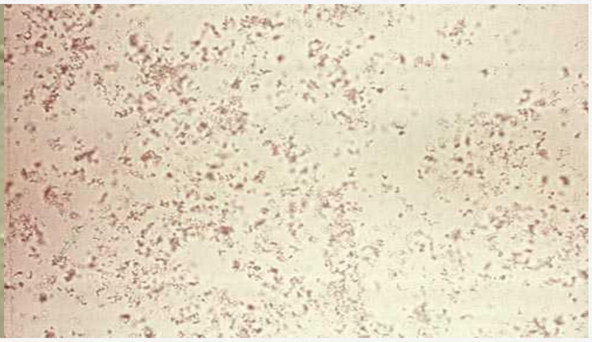
Amorphous urates

Amorphous urates of Na, K, Mg or Ca tend to form in acidic urine
Under the microscope, amorphous urates appear as yellow-brown mass of small rounded particles.

Amorphous phosphates

Amorphous phosphates are similar in general appearance, but tend to form in alkaline urine and appear as fine, colorless or slightly brown granules.



Difference between amorphous urate & phosphate

The distinction between amorphous urates and amorphous phosphates is often made on the urinary pH basis but the following can help in differentiation:

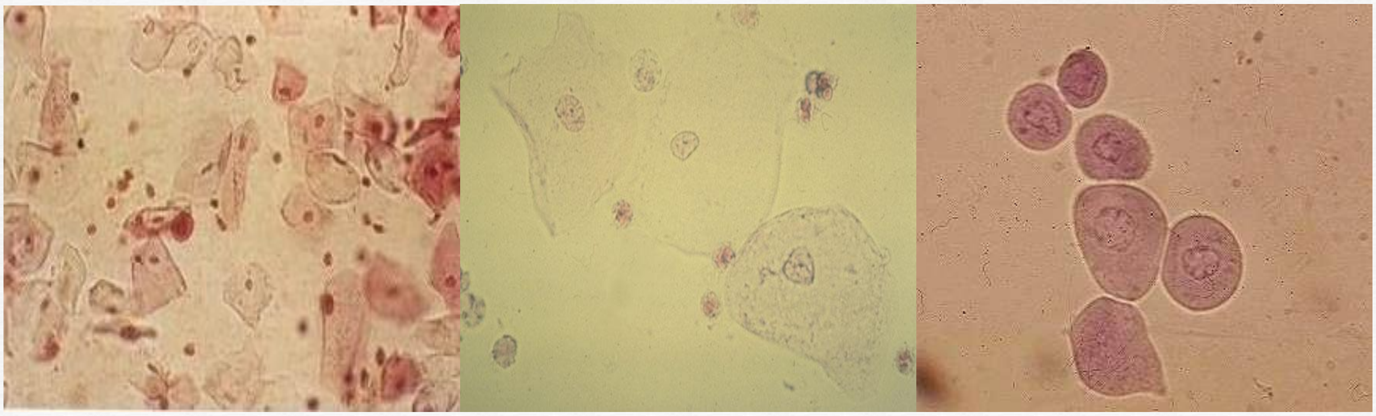
- **Examination of the centrifuge pellet**
  - The precipitate of phosphate is white
  - The precipitate of amorphous urate is pink (known as brick dust).
- **Precipitation**
  - Amorphous urates are precipitated by cooling (refrigeration)
  - Amorphous phosphate are precipitated by heating
- **Solubility**
  - Amorphous phosphate is soluble in acetic acid
  - Amorphous urates are insoluble in acetic acid (by acidification it converts to uric acid)

Urine Epithelial cells

Appearance

Urine epithelial cells are of three kinds: Renal tubule epithelial cells, Bladder epithelial cells, and Squamous epithelial cells

They are large (the largest cells which can be present in normal urine samples), flat cells with irregular borders, a single small nucleus, and abundant cytoplasm



Interpretation:

Epithelial cells in urine are generally of little specific diagnostic utility because:

Old cells lining the urinary tract at any level may continually slough into the urine.

Also in the case of voided samples, even cells from the genital tract can appear in the sample.

Increased number of epithelial cells associated with renal disease such as:

- Acute tubular necrosis
- Acute glomerulonephritis
- Pyelonephritis

> **Note:** *In cases of acute tubular necrosis, renal tubular epithelial cells containing large non lipid vacuoles may be seen, these are referred to as bubble cells. When lipids cross the glomerular membrane, the renal epithelial cells absorb the lipids and become highly refractive. These are called oval fat bodies and seen in cases of nephritic syndrome.*

Urine Ova

Trichomonas vaginalis

Habitat

If male: urinary bladder, urethra, prostate

If female: vagina, and cervix

Mode of infection

- Directly through sexual intercourse as parasite can't live outside body for long period
- Indirectly through using patients articles as under wear clothes

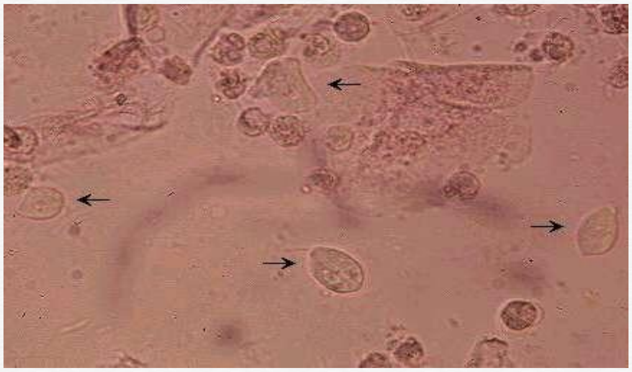
Pathology

Female symptoms

Infection is asymptomatic in 15-20% of cases

Symptoms associated with Trichomonas infection in women include:

- dysuria [painful urination]
- Painful sexual intercourse
- Burning sensation of the vagina



- Green/Yellow, frothy vaginal discharge with a strong foul-smelling odor.
- Trichomonas infection may cause Vaginitis , and Cervicitis.

Note: *Symptoms usually appear in women within 5 to 28 days of exposure.*

Male symptoms

Infection is asymptomatic in 50-90% of cases

Symptoms associated with Trichomonas infection in women include:

- dysuria
- Mild urethral discharge
- Slight burning after urination or ejaculation
- Trichomonas infection may cause Urethritis

Schistosome haematobium

Diagnosis

Clinical diagnosis Clinical signs & symptoms of urinary schistosomiasis include:

- Haematuria is a common finding.
- Proteinuria is frequently present.
- Eosinophils can often be found in the urine.
- There is usually also a blood eosinophilia.

Urine examination

Examination of urine for detection of egg this help in acute cases when egg can be detected easily in urine .

Urine other findings

Bacteriuria

Overview:

bacteriuria mean the presence of bacteria in urine

- Gram-negative bacilli Escherichia coli are the most common bacterium isolated from urine samples (>80% of UTIs are caused by E. coli).
- Smaller percent are caused by Gram-positive cocci (5% to 20%)

Appearance:

Bacteria can be identified in unstained urine sediments when present in sufficient numbers. Rod-shaped bacteria and chains of cocci can be found.

Note: *When urine is allowed to remain at room temperature, the number of bacteria doubles every 30 to 45 minutes.*

Interpretation:

Bacteria in the urine usually indicate a urinary tract infection (either cystitis or pyelonephritis),

> **Note:** *Bacteriuria of clinical significance is usually accompanied by pyuria in ~90% of cases.*

Urinary tract infection

A urinary tract infection (UTI) is a bacterial infection that affects any part of the urinary tract.
Any part of this system can become infected. As a rule, the farther up in the urinary tract the infection is located, the more serious it is.
Infection in the upper urinary tract generally affects the kidneys (***pyelonephritis***).
Infection in the lower urinary tract can affect the urethra (***urethritis***) or the bladder (***cystitis***).

causes

Urinary tract infections typically occur when bacteria enter the urinary tract through the urethra (an ascending infection) and begin to multiply in the bladder.
The majority of UTIs are believed to start in the bladder as a result of extreme contamination (commonly by Escherichia coli); but in many cases the initiating event is unclear.
In many cases, bacteria first travel to the urethra. When bacteria multiply, an infection can occur. An infection limited to the urethra is called urethritis. If bacteria move to the bladder and multiply, a bladder infection, called cystitis, results. If the infection is not treated promptly, bacteria may then travel further up the ureters to multiply and infect the kidneys. A kidney infection is called pyelonephritis.

> **Note:** *upper urinary tract infections like pyelonephritis may be hematogenous in origin.*

Risk factors

Some people appear to be more likely than are others to develop urinary tract infections. Risk factors include:

- Any abnormality of the urinary tract that obstructs the flow of urine (a kidney stone, for example and enlarged prostate gland can slow the flow of urine, thus raising the risk of infection.
- Being female. Half of all women will develop a urinary tract infection at some point during their lives, and many will experience more than one.
- Aging : after menopause, urinary tract infections may become more common because tissues of the vagina, urethra and the base of the bladder become thinner and more fragile due to loss of estrogen.
- Diabetes and other chronic illnesses that may impair the immune system.

Symptoms

Symptoms of bladder infection

dysuria : burning sensation throughout the urethra with urination
Frequent urination : an urge to urinate frequently but passing only small quantities of urine.
Nocturia: Need to urinate during the night.
Dribbling: inability to control urine release
Pyuria: Pus in the urine
Bacteriuria: Bacteria in the urine
Odour: Cloudy and foul-smelling urine

> **Note:** *kidney infection show the same symptoms as lower urinary tract infection beside Lower back, abdominal, or side pain that comes in waves, Night sweats and high fever*

Asymptomatic bacteriuria

Asymptomatic bacteriuria is a significant number of bacteria in the urine that occurs without any of the usual symptoms (burning during urination or increased frequency of urination).

Causes

Asymptomatic bacteriuria occurs in up to 6% of healthy individuals. It affects 18% of people with diabetes (mostly women), and 20% of elderly individuals (more often women than men). The reasons for the lack of symptoms are not well understood.

Although asymptomatic bacteriuria isn't causing any harm. but certain groups are at a higher risk for kidney infections if they develop asymptomatic bacteriuria. Those at risk include:

- People with diabetes
- Elderly people
- Pregnant women -- if asymptomatic bacteriuria is left untreated, up to 40% will develop a kidney infection.
- Patients with kidney stones

Note: *Most patients with asymptomatic bacteriuria do not need treatment because the bacteria isn't causing any harm. Treatment with antibiotics is recommended during pregnancy, because it significantly reduces symptomatic urinary tract infections, low birth weight, and preterm delivery.*

Mucus

Functions

Mucus forming cells are found scattered all over the urinary tract from the ascending section of the loop of Henle to the bladder so mucus can originate from the kidney or from the lower urinary tract. Mucus threads are made of ***Tamm-Horsfall protein fibrils***.

The exact function of mucus is unknown. Some think that this substance is a protection against bacterial infection. This action is done by coating the bacterial's pilis, essential to colonization of the lower urinary tract wall then the mucus coated bacteria are eliminated. Mucus can also protect the lower urinary tract against irritating chemical agents.

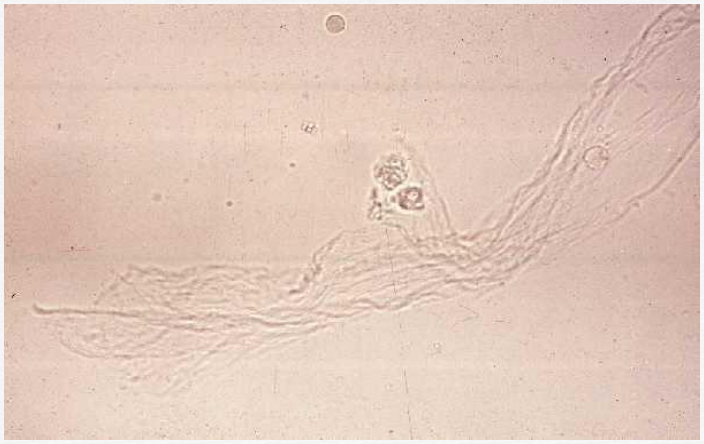
Appearance:

Mucus fibers are fibrillar and delicate. They have a very low refractive index.

Interpretation

Mucus is a frequent finding of the urinary sediment.

In the majority of cases, presence of mucus threads is a benign situation but sometimes irritating factor could stimulate mucus secretion.



Note: *Mucus originating from the kidney is made of Tamm-Horsfall protein. This explains the frequent association of mucus threads and casts.*

Note: *the presence in male is normal due to increased secretion of cowper gland*

Sperm in urine (spermatorroea)

They may be seen in urine from males collected by voiding.

Interpretation

Urinary spermatozoa are a contamination arising from sexual activity. With a male subject, these represent a residual drainage.

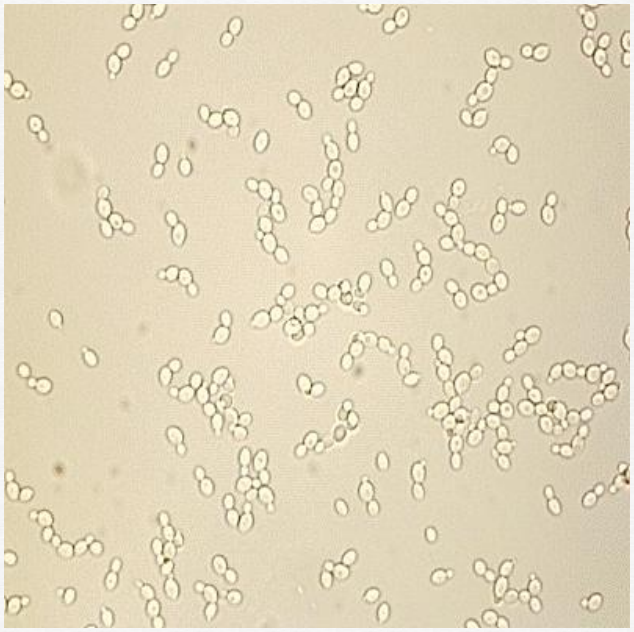
They may present in urine of men after epileptic convulsions or nocturnal emission.

Also may be found in case of prostatitis and orchitis.

Note: *Rarely, they may be observed in voided urine from a recently-bred female (a vaginal contamination).*

Yeast

Appearance:



Yeasts in unstained urine sediments are round to oval in shape, colorless, and may have obvious budding

They are often difficult to distinguish from red cells and amorphous crystals but are distinguished by their tendency to bud.

Interpretation

the presence of yeast may be the result of a contamination with vaginal secretion.

Yeast cells may represent a true yeast infection most often they are ***Candida albicans***., which may colonize bladder, urethra, or vagina.

Yeasts are often observed in specimens that contain sugar. It is important to be careful with these specimens because a yeast infection is a frequent finding with diabetic patients.

Complete stool analysis

What is the stool analysis test?

A stool analysis is a series of tests done on a stool (feces) sample to help diagnose certain conditions affecting the digestive tract.

Why It Is Done

- Help identify diseases of the digestive tract, liver, and pancreas.
- Help find the cause of symptoms affecting the digestive tract, including prolonged diarrhea, bloody diarrhea, an increased amount of gas, nausea, vomiting, loss of appetite, bloating, abdominal pain and cramping, and fever.
- Look for intestinal parasites.
- Look for the cause of an infection, such as bacteria, a fungus, or a virus.
- Check for poor absorption of nutrients by the digestive tract (malabsorption syndrome).

Stool analysis may be done for these patients:

- Patient with abdominal pain or abdominal discomfort
- Patient with diarrhea
- Patient with anemia
- Patient who is too thin or do not grow well
- Patient with stool color that is changed to abnormal color
- Patient with skin disease as Urticaria which may due to parasitic infection with helminthes.

Preparation methods

Direct saline wet mount

- Place a drop of saline on the slide.
- Pick up a small amount of fecal material on the end of an applicator stick.
- Emulsify in the saline and cover with a cover slip.
- Examine on low and high power.
- The entire preparation must be examined for the presence of eggs, larvae and protozoa.

Note: *Take small amounts of material from several different areas (stool surface and deep inside), especially from bloody and/or mucoid areas.*

Saline sedimentation method

- add saline to the stool container.
- Mix well in the container
- Pour the solution from the container into a tube
- Leave the tube to stand in the rack for 20-30 minutes
- Examine the stool precipitate under microscope.

Note: *This method is suitable for parasites that such as Ascaris lumbricoides , Shistosoma mansoni, Fasciola gigantica ,Strongyloides stercoralis*

احتياطات تجميع و تخزين و تحضير عينه البراز

التجميع:

1. لابد من أخد كمية كافيه من البراز
2. لابد من وجود وعاء نظيف للتجميع
3. يجب وضع بيانات صاحب العينة علي الوعاء (على الغطاء و على الكوب نفسه)
4. عينة الصباح الباكر هي الأفضل (لان البراز هنا مجمع طوال الليل و فرصه ظهور الطفيليات و البويضات اكبر)
5. يفضل الامتناع عن أخد بعض الأدويه المعينة قبل أجراء التحليل مثل: (مضادات الاسهال و مضادات الديدان و الملينات)
6. مراعاة عدم اختلاط البول بالبراز خاصة عند الأطفال لأنها تقتل الأطوار النشطة
7. مراعاة عدم اختلاط البراز بالدم عند النساء أثناء الدورة الشهرية حتى لا يكون التحليل ثم التشخيص خطأ
8. ترفض العينة من الشخص الذي أجرى أشعة بالصبغة خلال يومين بعد الأشعة
9. عينة الأطفال فى الحافظات ترفض إذا كان البراز جاف نتيجة تشرب الحفاظ .
10. إذا أخذت العينة خارج المعمل يفضل إعطاء العينة إلى المعمل فى فترة لا تزيد عن الساعه منعاً لتحلل بعض الطفيليات
11. لا تقبل عينات البراز من خارج المعمل إذا كانت في علبة كريم أو زبادي
12. أثبتت الأبحاث أنه يجب فحص ثلاثة عينات براز على الأقل فى ثلاثة أيام متتالية حيث أن الأعتماد على عينة واحدة فقط لا يكفى وذلك لزيادة الفرصة فى رؤية الطفيليات

التخزين:

1. يفضل فحص العينة فى الحال (خلال ساعة من التبرز) وذلك لزيادة الفرصة لرؤية الأطوار النشطة قبل موتها
2. تجنب ترك كوب العينة معرضا للهواء حتى لا تجف العينة مما يؤدى إلى قتل الأطوار النشطة و اليرقات و حتى لا تتلوث العينة بطفيليات الجو الخارجي(***coprozoic protozoa***).
3. إذا كان ضرورة تخزين البراز فيتم تحضيره (بالترسيب) أولا ثم يخزن بالثلاجة عند درجة 4 مئوية لكن لاحظ ألا يمر عليها وقت طويل (24 ساعة) حيث أن الأطوار النشطة تموت وكذلك الأطوار المتحوصلة تتحلل و تختفي.

التحضير:

1. يفضل أن يتم التحضير بواسطة الشخص الذي سيقوم بعمل بالتحليل حتى يتثنى له القيام بالفحص الظاهري أثناء التحضير.
2. إذا تعزر ذلك لابد من سؤال من حضر العينة عن كل البيانات الخاصة بالفحص الظاهري
3. يتم تحضير العينة فى أسرع وقت ثم تخزينها فى الثلاجة بعد ذلك إذا لم تفحص فى الحال.
4. إذا كان بعينة البراز أى شيء غير طبيعي كالدم مثلا أو صديد أو مخاط يفضل فحص ذلك الجزء من العينة أولا تحت الميكروسكوب قبل البدء فى تحضير العينة.
5. إذا كان لديك أكثر من عينه براز فى نفس الوقت افحص أولا العينات المحتوية على مخاط أو دم ثم العينات السائلة و فى النهاية افحص العينات المتماسكة (العينات التي بها مخاط أو السائلة احتمال وجود طفيل نشط فيها أكثر من المتماسكة).

Gross examination

Color

Normal:

Brown color is the normal color of stool

Why stool color is brown? The characteristic brown color of feces is due to *stercobilin* and *urobinin*, both of which are produced by bacterial degradation of bilirubin.

Abnormal:

Black color: indicate iron medication (for treatment of anemia) or upper GIT bleeding (due to peptic ulcer, stomach carcinoma or esophageal varices).

> **Note:** *The black color is caused by oxidation of the iron in the blood's hemoglobin.*

Bright red color (Hematochezia): indicate lower GIT bleeding (due to piles and anal fissure).

Clay color (gray-white): indicate obstructive jaundice

Pale brown color: with a greasy consistency indicate pancreatic deficiency causing malabsorption of fat (often with offensive odor).

Yellow-green color: occurs in the stool of breast-fed infants who lack normal intestinal flora (low bil. conversion) and may also occurs due to rapid transit of feces through the intestines.

Red brown color: indicate drugs as Tetracyclines, and Rifambicin antibiotics

> **Note:** *if stool color is black and there is no history of iron medication FOBT is recommended.*

Odour

Normal:

Normally offensive

Why stool odour is offensive? Fecal odor results from gases produced by bacterial metabolism, including skatole, mercaptans, indoleand hydrogen sulfide formed by bacterial fermentation and putrefaction.

> **Note:** *Consumption of foods with spices may result in the spices being undigested and adding to the odor of feces.*

Abnormal:

Very offensive: usually seen in cases of constipation and with certain types of food that produce excessive gases, Bacterial infection and malabsorption.

Foul-smelling stool: are characteristic of steatorrhea.

pH (reaction)

Normal:

Normally variable and diet dependent and is based on bacterial fermentation in the small intestine.

Why stool pH is variable? Because stool pH mainly depends on the type of diet.

Abnormal:

High alkaline stool

Physiological

- High protein diet

Pathological

- Secretory diarrhea
- Colitis
- Antibiotic use (impaired colonic fermentation)

High acidic stool

Physiological

- High carbohydrate diet

Pathological

- Poor fat absorption
- Poor absorption of sugars as in lactose intolerance

> **Note:** *Breast-fed infants have slightly acid stool; bottle-fed infants have slightly alkaline stools.*

> **Note:** *Stool Reducing Substances Test is recommended if lactase deficiency is suspected*

Consistency

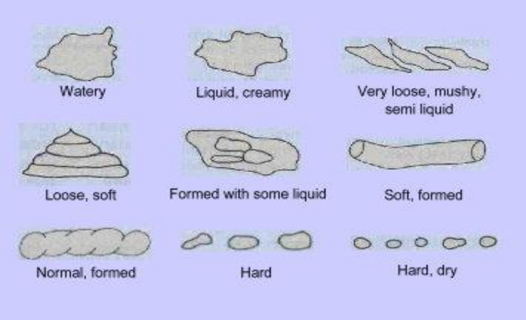
Normal:

Normally well formed

Abnormal:

Abnormal consistency may be graded as follow:

Very hard: seen in cases of constipation
Semi formed: seen in the cases of parasitic infection
Soft: seen in the cases of parasitic infection
Loose: seen in the cases of diarrhea
Watery: mostly seen in cases of bacterial infection



Mucus

Normal:

Normally undetectable amount produce by GIT and found in the stool.

Abnormal:

Abnormal mucus in the sample appears as white patches and according to the amount of mucus it can be graded using signs (+, ++, +++)

Mucus abnormally can be found in the stool in the following cases:

- Excessive straining at stool
- Spastic colon (translucent mucus on the surface of stool)
- Ulcerative colitis
- Bacillary dysentery (mucus with fresh pus)
- Amoebic dysentery (mucus with fresh blood)

Appearance pus

Normal:

Normally not found. (you cannot see it by naked eye).

Abnormal:

Usually detected with mucus and appear as white patches in the stool, it indicate ulcerative colitis or bacterial infection as *bacillary dysentery*.
Also it can be graded using signs (+, ++, +++).
Presence of detectable pus by naked eye means that the microscopic pus must be over 100.

ملحوظة : إذا كان الصديد أو المخاط غير واضح بالفحص الظاهري أو أن تحضير العينة قد تم قبل أن تسجل الفحص الظاهري فاعتمد على الفحص الميكروسكوبي لكتابه الصديد أو المخاط في الفحص الظاهري حيث إذا وجدت صديد تحت الميكروسكوب أعلى من 80 اكتب الصديد و المخاط فى الفحص الظاهري ++ أو +++

Gross blood

Normal:

Normally no blood seen in the stool (you cannot see it by naked eye).

Abnormal:

*Abnormal fresh blood (**Hematochezia**) seen in cases of lower GIT bleeding and also known as "bright red blood per rectum" and abbreviated **BRBPR**.*

> **Note:** *It is distinguished from melena, which is stool with blood that has been altered by the gut flora and appears black*

Causes:

blood that is mixed with stool

- ulcerative colitis
- colorectal cancer

blood that is not mixed with stool

- Bilharzias (schistosoma mansoni infection)
- anal fissure
- piles

blood with diarrhea and mucus

- amoebic dysentery

ملحوظه : إذا وجدت دم فى العينة بالفحص الظاهري فلابد من كتابه RBCs في الفحص الميكروسكوبي أكثر من 100 (over 100).

Naked eye parasite

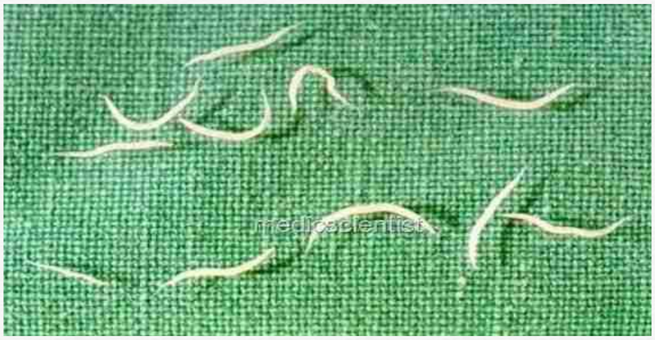
Normal: no parasites or larva appear in the stool

Abnormal: In some cases the whole worm or parts of its body appear in the stool and can be seen by naked eye.

Two worms can be seen by naked eye in the stool:

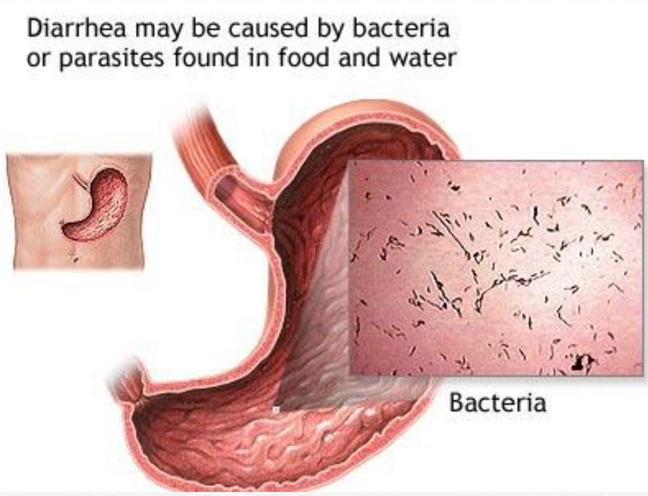
Ascaris lumbericoides

Entrobius vermicularis



Diarrhea

Diarrhea is an increase in the volume of stool or frequency of defecation (having three or more loose or liquid bowel movements per day). It is one of the most common clinical signs of gastrointestinal disease.



Note: *The loss of fluids through diarrhea can cause dehydration and electrolyte imbalances.*

Types of diarrhea

There are numerous causes of diarrhea, but in almost all cases, this disorder is a manifestation of one of the four basic mechanisms.

Secretory diarrhea

Secretory diarrhea means that there is an increase in the active secretion, or there is an inhibition of absorption. The most common cause of this type of diarrhea is bacterial toxins such that associated with cholera.

In addition to bacterial toxins, a large number of other agents can induce secretory diarrhea by turning on the intestinal secretory machinery, including:

hormones secreted by certain types of tumors (e.g. vasoactive intestinal peptide)

a broad range of drugs (e.g. some types of asthma medications, antidepressants, cardiac drugs)

Osmotic diarrhea

Osmotic diarrhea occurs when too much water is drawn into the bowels.

Osmotic diarrhea typically results from one of two situations:

Ingestion of a poorly absorbed substrate: as carbohydrate or divalent ion.

Malabsorption: A common example of malabsorption, afflicting many adults humans and pets is lactose intolerance resulting from a deficiency in the brush border enzyme lactase.

Motility-related diarrhea

Motility-related diarrhea is caused by the rapid movement of food through the intestines (hypermotility). If the food moves too quickly through the GI tract, there is not enough time for sufficient nutrients and water to be absorbed. This can be due to a diabetic neuropathy, as a complication of menstruation or Hyperthyroidism which can produce hypermotility.

In order for nutrients and water to be efficiently absorbed, the intestinal contents must be adequately exposed to the mucosal epithelium and retained long enough to allow absorption. Disorders in motility than accelerate transit time could decrease absorption, resulting in diarrhea even if the absorptive process per se was proceeding properly.

Inflammatory diarrhea

Inflammatory diarrhea occurs when there is damage to the mucosal lining or brush border, which leads to a passive loss of protein-rich fluids, and a decreased ability to absorb these lost fluids. It can be caused by bacterial infections as Salmonella , viral infections as rotaviruses, parasitic infections as Giardia, or autoimmune problems such as inflammatory bowel diseases.

Microscopic examination

احتياطات تحضير و فحص عينه البراز

1. يجب التأكد أن الميكروسكوب صالح للعمل من حيث سلامة ونظافة العدسات ومن حيث الإضاءة والمسرح
2. التأكد من نظافة الشرائح الميكروسكوبية وكذلك غطاء الشرائح.

3. تحضير شريحة نموذجية (*not too thin not too thick*) هذه الشريحة تمكنك م
مجلة إذا وضعت فوقها.
4. ابدأ الفحص من نقطة معينة على الشريحة و اتبع خط سير محدد zigzag
5. حاول أن تفحص الشريحة بطريقة متداخلة overlapping حتى يتم فحص الشريحة كاملة دون ترك فراغات.
6. افحص شريحتين على الأقل لكل عينة و إذا كنت تستخدم طريقه اللطخه اختار عينه من أماكن مختلفة حتى يمكن الحكم علي نتيجة العينة جيدا.
7. عند وجود صديد أو دم فى الشريحة افحص أكثر من شريحتين للتأكد التام من عدم وجود ديدان
8. لا تضع فى تفكيرك شيء معين و أنت تبحث فى العينة مثل أن تبحث عن ova معينه مثلا
9. يفضل استخدام العينين فى الفحص (ميكروسكوب binocular) حتى لا يصاب الشخص الذي يفحص بالصداع إذا استخدم عين واحدة
10. البراز يحتوى على أشكال كثيرة جدا للطعام الغير المهضوم لدلك لابد أن يكون عندك الخبرة للتفريق بين الأشكال الطبيعية و البويضات.
11. عند الشك فى احتمال وجود بويضة ما لا تعرفها اترك العينة لغيرك يفحصها أو استعين بالصور من الكتاب أو الكمبيوتر.
12. بعض الأعراض المرضية على المريض تمكنك من التأكد من صحة تحليلك و توقع الطفيل الموجود فى العينة.
13. ليس شرطا وجود طفيليات بالبراز لتشخيص مشكلة المريض بل أن احد وسائل التشخيص هي استبعاد وجود طفيليات لذلك عند الفحص الجيد جدا للعينة و المتأني و بعد التأكد من عدم وجود بويضات أوlarva بها اكتب التحليل بدون طفيليات.
14. ابدأ فحص الشريحة كلها بالعدسة ال 10 أولا ثم بعد ذلك افحص بالعدسة ال 40 ما تشك فيه و كذلك للتأكد من الأطوار النشطة حيث أنها تظهر أوضح بالعدسة ال40

Different Cells in stool

RBCs

Appearance:

تظهر كرات الدم الحمراء تحت الميكروسكوب كخليه صغيره الحجم (اصغر من خليه الصديد) و تبدو بغشاء مزدوج (لكن في الحقيقة غشائها ليس مزدوج و إنما يظهر هكذا نتيجة امتلائها بالهيموجلوبين الذي يعطى هدا الانطباع).

ملحوظة

- وجود دم فى البراز يحدث فى الغالب نتيجة نزيف و يظهر أيضا أثناء الفحص الظاهري مما يؤكد الرؤية تحت الميكروسكوب
- لون كرات الدم الحمراء تحت الميكروسكوب ليس احمر بل لا لون لها و فى بعض الأحيان تظهر تحت الميكروسكوب شوائب حمراء على شكل لطخه أو خط طويل و هي ليس دم أبدا بل هي ألياف من الملابس أو من الأكل خاصة البطيخ و الطماطم.

Normal:

Normally few amounts of RBCs (0-3) are seen under high power field of microscope.

Counting:

RBCs are counted under HPF of microscope and the range of count is written for example 10-12 or 30-40 and so on.

Interpretation: The same causes as in blood gross

PUS

Appearance:

تظهر كرات الدم البيضاء أو الصديد تحت الميكروسكوب كخليه كبيرة الحجم و بها نواه .

Normal:

Normally few amounts of pus (0-5) are seen under high power field of microscope.

ملحوظة

- الصديد دائما يوجد فى تجمعات
- فى بعض الأحيان تكون العينة كلها صديد و دلك فى حالات الإصابات البكتيرية و هنا لاحظ انه لا توجد ماده برازيه فلا يمكن الحكم على حاله الهضم.
- ابحث دائما عن الأماكن التي تظهر بيضاء بالعين المجردة على الشريحة لأنها غالبا ما تكون مخاط و عليه تجمع من الصديد .

Counting:

Pus is counted under HPF of microscope and the range of count is written as in the case of RBCs.

Note: *if pus count is over 100 stool culture is recommended.*

Interpretation:

Abnormal pus under microscope is seen in:

- Bacterial infection as Shigellosis , Salmonellosis
- Inflammation of the intestines, such as Ulcerative colitis

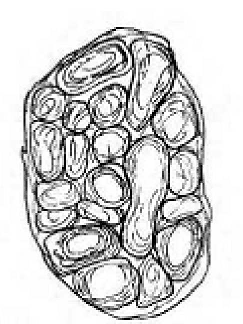
Note: *Usually pus is absent or few in cases of:*

- *Viral diarrheas (Rotavirus infection)*
- *Parasitic infestations (e.g. Giardia, Entamoeba)*
- *In nonspecific diarrhea (e.g. drug or food induced).*

Note: *in cases of semi formed stool with pus and RBCs , widal test is recommended specially if the patients suffers from fever*

Digestive state

Starch



Appearance:
يظهر من تناول اطعمة مثل القمح و الارز و وله اشكال قياسية و لكن لا توجد بشكل كبير فى الحقيقة بل تكون على هيئة شكل عشوائى و تأخذ اللون البنى المميز للبراز.

Normal:
Normally found in the stool and graded as (+) when seen under HPF.

Interpretation:
Increased starch in stool (++ or +++) indicate a case of ***indigestion***.

Dyspepsia (indigestion)

Dyspepsia (Indigestion) is a medical condition characterized by indigestion with chronic or recurrent pain in the upper abdomen, upper abdominal fullness and feeling full earlier than expected with eating. It can be accompanied by bloating, belching, nausea or heartburn.

symptoms

The characteristic symptoms of dyspepsia are upper abdominal pain, bloating, fullness and tenderness on palpation.

Pain worsened by exertion and associated with nausea and perspiration may also indicate angina.

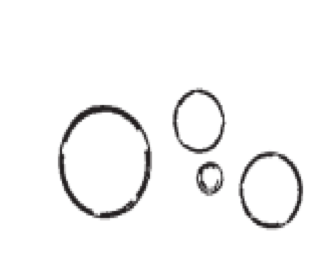
> **Note:** *Pain worsened by exertion and associated with nausea and sweating may also indicate angina.*

causes

- over eating , spicy food , too fast eating , drinking water during eating due to dilution of digestive enzymes , alcohol drug eat ,stress , and peptic ulcers
- Occasionally dyspeptic symptoms are caused by medication, such as calcium antagonists (used for angina or high blood pressure), nitrates (used for angina), theophylline (used for chronic lung disease), corticosteroids and non-steroidal anti-inflammatory drugs.

Fat

Appearance:
يظهر من تناول الدهون فى الطعام سواء كانت من مصدر نباتى او مصدر حيوانى و له احجام مختلفة تشبه الكرستالات تأخذ اللون الاصفر البرتقالى .



Normal:
Normally found in the stool and graded as (+) when seen under HPF.

Interpretation:
Increased fat in stool may due to Malabsorption :

Malabsorption is a state arising from abnormality in digestion or absorption of food nutrients across the gastrointestinal (GI) tract.

Steatorrhea (fat malabsorption)

Steatorrhea (or steatorrhoea) is the presence of excess fat in feces. Stools may also float due to excess lipid, have an oily appearance and be especially foul-smelling.

causes

- lack of bile acids due to:
  - *liver damage*
  - *Hypolipidemic drugs*
  - *Gallbladder removal (cholecystectomy)*
  - *choledocholithiasis (obstruction of the bile duct by a gallstone)*
  - *pancreatic cancer (if it obstructs biliary outflow)*
  - *Cholangiocarcinoma (cancer of the biliary tracts or gallbladder)*
- Defects in pancreatic juices (enzymes)
- giardiasis (a protozoan parasite infection)
- Inflammation of the lining of the intestines, which may occur with conditions such as ulcerative colitis or Crohn's disease.

  Note: ***Orlistat*** *(also known by trade names* ***Xenical****) is a diet pill (Weight-loss drugs) that works by blocking the enzymes that digest fat. As a result, some fat cannot be absorbed from the gut and is excreted in the feces instead of being metabolically digested*

Vegetable cells

Appearance:

يظهر من تناول الاطعمة الغنية بالالياف مثل الخس.
لاحظ انها تشبه البويضات لدلك تحتاج خبرة للتفريق بينها وبين البويضات و عامة اذا وجدت الخلايا الخضرية توجد بكمية كبيرة اكثر بكثير من تواجد اى بويضات فى العينة.

Normal:
Normally found in the stool and graded as (+) when seen under HPF.

Interpretation:
Increased vegetable cells in stool have no clinical significance and considered as residual food.

Muscle fibers

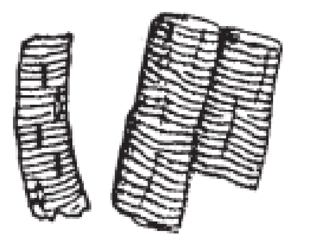
Appearance:

تظهر من تناول اللحوم و شكلها مميز وواضح(مثل شكل الحصيره) ويمكن تقديرها بسهولة.

ملحوظه

براز الاطفال الرضع فى اول 6 اشهر لايحتوى على *muscle fibers* لأنـه لا يأكل طعام طبيعي و لدلك تكتب *absent* أما بعد 6 أشهر فيأكل الطفل بصورة طبيعية ويكون البراز مثل الشخص العادي.

Normal:
Normally found in the stool and graded as (+) when seen under HPF.



Interpretation:
Increased muscle fibers in stool have no clinical significance and considered as residual food.

Note: *The presence of large amount of undigested meat fibers may be caused by pancreatitis*

Parasites

What are the types of parasites that attack human?

Protozoa: are microorganisms classified as unicellular eukaryotes

Helminthes: or ***Parasitic worms***, a division of eukaryotic parasites that live inside their host. They are worm-like organisms that live and feed off living hosts.

What is the forms of protozoa that can appears in stool?

Trophozoite: metabolically active invasive stage.

Cyst: "vegetative" inactive form resistant to unfavorable environmental conditions they are refractile and more easily detected unstained.

In saline preparations protozoan cysts can be recognized as refractile bodies (shine brightly when focused). Cysts can be identified by their shape, size, nuclei, and inclusions as seen in an iodine preparation.

What is the forms of helminthes that ca appears in stool?

Ova: that is results from sexual reproduction of helminthes.

Eggs are recognized by their:

- size,
- color (colorless, pale yellow, brown),
- morphological features.

Larva: the stage that is come from the ova.

In a fresh faecal specimen, S. stercoralis is the only larva that will be found. It can be easily detected in a saline preparation by its motility and large size.

ملحوظه : يمكن تقدير شده الاصابه باستخدام الإشارات + و ++ و +++ بجوار اسم الطفيل او استخدام الكلمات (many, Scanty few) لكن لاحظ أن الاصابه الخفيفة أو الشديدة علاجها واحد فلابد للمريض من اخذ الجرعة العلاجية كاملة.

What are the limitations of stool examination in the diagnosis of gastro-intestinal parasites?

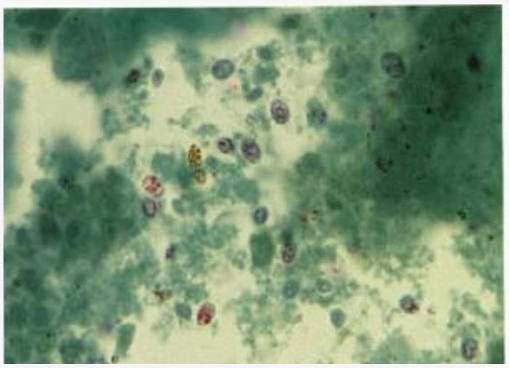
1. The demonstration of parasite eggs or larvae in the stool provides positive evidence that an animal is infected but does not indicate the degree of an infection.
2. The failure to demonstrate eggs or larvae does not necessarily mean that no parasites are present; they may be present in an immature stage or the test used may not be sufficiently sensitive.
3. Various factors can limit the accuracy and significance of a stool egg count.
   - There is a fairly regular fluctuation in faecal egg output.
   - Eggs are not evenly distributed throughout the faeces.
   - The quantity of faeces passed will affect the number of eggs per unit weight.
   - The egg output is influenced by the season of the year (large infections may be acquired during rainy seasons).
   - Eggs may not be detected due to low numbers of them or to low test sensitivity.

Other findings

Normal constituents of stool

Bacteria: normally non pathogenic bacteria are found in the stool and usually bacilli such as *Escherichia coli* and *Lactobacillus sp*.

> **Note:** *pathogenic bacteria may be found in stool (such as Salmonella, Shigella, and Staphylococcus aureus) and this will leads to pus formation, stool culture can differentiate between pathogenic and non pathogenic bacteria.*



Yeast: normally stool contain harmless yeast cells such as *blastocystis hominis*

Crystals: normally stool contain triple phosphate, calcium oxalate and cholesterol crystal due to food ingestion.

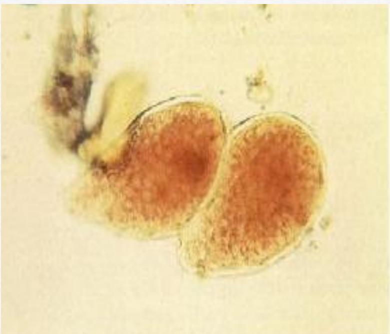
Fibers: normally stool contains many fibers that may arise from clothes or undigested plant food.

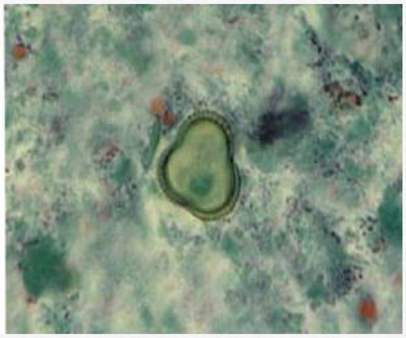
Air bubbles: normally found and has no clinical significance.

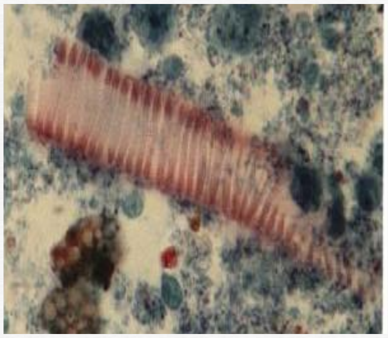
Oil droplets: normally found, they are very bright and completely rounded.

> **Note:** *some times oil droplets are muddled with cysts but cysts are not so bright and not so rounded and if you stain the smear with iodine nucleus of cyst will be seen.*

Undigested food: normally found, it has many shapes as pollen grain , bundle of vegetables ,plant cells.







Abnormal constituents of stool

Charcot-Leyden crystals:

Formation

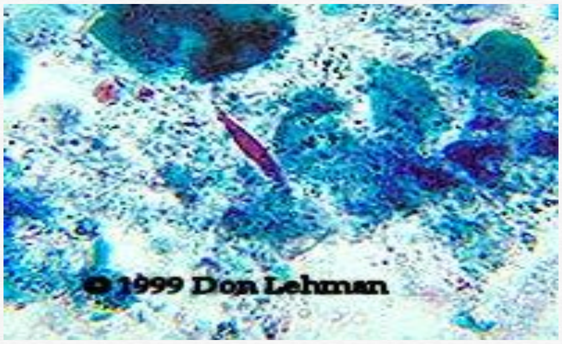
Crystals are formed from the breakdown of eosinophils and consist of the enzyme lysophospholipase, synthesized by eosinophils

Appearance

Crystals are slender and pointed at both ends, consisting of a pair of hexagonal pyramids joined at their bases.

Clinical significance

Sometimes found in parasitic infestation (especially amebiasis and ascariasis) and in cases of ulcerative colitis.



Fecal Occult Blood

Faecal occult blood

When the bleeding is chronic with only small amounts of blood being passed in the faeces, the blood (or its breakdown products) is not recognized in the faeces and is referred to as occult (hidden) blood.

An average, healthy person passes up to 2.0 mL of blood per 150 g of stool into the GI tract daily. Passage of more than 2.0 mL of blood in the stool in 24 hours is pathologically significant.

> **Note:** *Normal stool color don't rules out the presence of occult blood where black color need more than 2 ml blood to be passes in stool*

> **Note:** *FOBT is a screening test so more tests will need to be done to diagnose the cause of the bleeding.*

Aim of test

- FOBT may be used to check for occult blood in cases of black stool (melena) that are associated with upper gastrointestinal hemorrhage.
- FOBT is used to check for the cause of anemia specially iron defcancy anemia.
- FOBT is a useful tool to screen for early colorectal cancer because tissue with cancer changes is more likely to bleed than normal colon tissue.

> **Note:** *A negative test does not necessarily mean there are no colorectal diseases present. Not all cancers bleed, and not all cancers bleed all the time. That is why it is so important to continue to screen each year for colon cancer.*

> **Note:** *the fecal occult blood test is recommended yearly for everyone starting at age 50 to allow for the early detection and prevention of colorectal cancer.*

What is the cause of black color of stool in case of melena?

Generally GIT bleeding can be divided into upper GIT bleeding and lower GIT bleeding, Lower gastrointestinal bleeding, commonly abbreviated LGIB, refers to any form of bleeding distal to the ligament of Treitz. This includes the last 1/4 of the duodenum and the entire area of the jejunum, ileum, colon, rectum, and anus. Upper gastrointestinal bleeding (UGIB) refers to bleeding above the duodenum.

Typically, bleeding from the upper gastrointestinal tract manifests as black or tarry stool known as melena. The black color develops because of chemical changes that occur as the blood passes through the digestive tract (*oxidation of the iron in hemoglobin during its passage through* GIT).

Methods of detection

Two methods can be used for detection of stool occult blood, the conventional faecal occult blood tests look for heme while the newer, modern tests look for globin.

Chemical method (cFOBT)

Sample required

Random stool sample can be used for the test (only a small sample for smearing is necessary)

> **Note:** *Since colorectal cancers do not bleed all the time, the test for blood in the stool is done over several days on three different stool samples. This increases the chance of finding blood in your stool.*

Preparation of the patient

Before doing FOBT by the chemical method, avoid the following for 2 to 3 days before the test:

- Foods that can cause the test to be positive for blood as beets ,mushrooms, broccoli ,bean sprouts, apples Carrots and melon. (sources of peroxidase activity)
- Red meat, which contain hemoglobin, myoglobin, and certain enzymes that can give false test results
- Iron medication that is used for treatment of anemia
- Aspirin (or products that contain aspirin) and nonsteroidal anti-inflammatory drugs (NSAIDs) or other medicines that irritate the stomach or intestines
- Vitamin C supplements or beverages in excess of 250 mg/day
- Do not do the test during your menstrual period or if you have active bleeding from hemorrhoids.
- Do not do the test if you have bleeding gums, or nosebleeds.

Principle

In 1864, Van Deen first developed a chemical method, to detect occult blood.

The test is based on the principle that haemoglobin and its derivatives react in a similar way to peroxidase enzymes, i.e. they catalyze the transfer of an oxygen atom from a peroxide such as hydrogen peroxide to a chromogen such as guaiacum (extracted from trees in the genus Guaiacum), Benzidine , o-tolidine or aminophenazone. Oxidation of the chromogen is shown by the production of a blue or blue-green colour.

The chemical reaction involved is as follows:

$$\text{Hb} + 2\ H_2O_2 \longrightarrow 2H_2O + \text{oxygen free radicals}$$

Oxygen free radicals + ***chromogen*** (Colorless) ⟶ Oxidized ***chromogen*** (Blue color)

Sensitivity

This method is capable of detection of 6 mg of hemoglobin per gram of feces in 90% of observations, but will fail 80% of the time to detect up to 1.5 mg/g of feces.

Disadvantages:

- Lack sensitivity to small amounts of blood and might fail to detect slow rates of blood loss.
- Inability to distinguish human heme from heme present in many foods, such as dietary meats
- Non specific to colon bleeding because it based on detection of heme present in the stool, which can originate from bleeding anywhere in the gastrointestinal tract
- False-positive results are common and may cause unwarranted anxiety about cancer and lead to unnecessary further tests.
- False-negative results are also common and may miss a cancer in its early stages.

Results

Normal [negative] result

A normal test (no color change) means that there was no blood in stool at the time of the test.

Abnormal [positive] result

An abnormal test (blue color change) means that there was some blood in stool at the time of the test.

Note: *The strength of the colour formed depends on the concentration of hemoglobin in the sample.*

False-positive result

- Recent ingestion of under-cooked meats that contain hemoglobin, myoglobin or foods that contain a sources of peroxidase activity
- swallowed blood due to bleeding gums, coughing up blood or nosebleeds
- Iron medications
- Use of aspirin or non steroidal anti-inflammatory drugs (NSAIDs)

False negatives result

- vitamin C supplements which acts as antioxidants.
- False negative results may occur when occult blood is not uniformly distributed throughout the stool.

Immunochemical method (iFOBT)

Principle

In 1974, Adams first developed an immunological based method for the detection of occult blood that utilized an antigen-antibody reaction between human hemoglobin and anti-human hemoglobin antibody.
Immunochemical fecal occult blood tests utilize anti-human hemoglobin antibodies attached to a solid phase to detect the globin portion of un-degraded human hemoglobin in stool.

Sample required

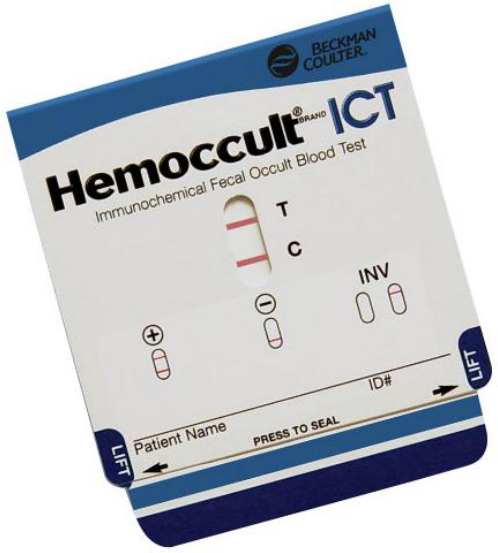
Random stool sample can be used for the test (only a small sample is necessary)

Preparation of the patient

No special drug or dietary restrictions are required. Results of this test are not affected by dietary peroxidases, animal blood, or vitamin C.

Advantage

These tests detect the globin in feces rather than heme. By detecting globin the tests are more specific for lower gastrointestinal bleeding. (hemoglobin from the upper gastrointestinal tract is mostly degraded by bacterial and digestive enzymes before reaching the large intestine, hemoglobin from lower gastrointestinal tract bleeding undergoes less degradation and remains immunochemically reactive)
Immunochemical fecal occult blood tests is specific to human globulin so it do not react with animal hemoglobin, peroxidase compounds, or with drugs, vitamins or other substances that can produce false results.



Sensitivity

The test is very sensitive -- it is capable of detection as little as 0.3 mg of human hemoglobin per gram of feces

Results

Normal [negative] result

After exactly five minutes one red band appears in the control zone

Abnormal [positive] result

After exactly five minutes two red bands appears one in the control zone and one in the test zone

Interpretation of +ve results

Conditions that can cause blood occult in the stool include:

Colorectal cancer

Colorectal cancer, also called colon cancer, includes cancerous growths in the colon, rectum and appendix.

Peptic ulcers

A peptic ulcer is an ulcer (defined as mucosal erosions equal to or greater than 0.5 cm) of an area of the gastrointestinal tract that is usually acidic and thus extremely painful.

Ulcerative colitis

Ulcerative colitis is a form of inflammatory bowel disease (IBD), a disease of the intestine, specifically the large intestine or colon, that includes characteristic ulcers, or open sores, in the colon.

Crohn's disease

Crohn's disease (also known as regional enteritis) is a chronic, episodic, inflammatory bowel disease (IBD) and is generally classified as an autoimmune disease. It can affect any part of the gastrointestinal tract from mouth to anus;

Esophageal varices

Bleeding esophageal varices occur when veins in the walls of the lower part of the esophagus and sometimes the upper part of the stomach is wider than normal (dilated)., it is cased by increased blood pressure in the portal vein caused by liver disease (portal hypertension).

Gastric carcinoma

Stomach or gastric cancer can develop in any part of the stomach and may spread throughout the stomach and to other organs; particularly the esophagus, lungs and the liver.

Gastritis

Gastritis is an inflammation (swelling) of the lining of the stomach. The Most common causes are: Smoking , Alcohol , Infection of the stomach with *Helicobacter pylori* bacteria and Medications (such as aspirin or anti-inflammatory drugs)

Infectious diseases

One of the commonest causes of positive occult blood testsin tropical and other developing countries are hook worm infection, whipworm infection, and strongyloidiasis.

Parasitology assays

Entameoba histolytica

Mode of infection

- Amoebiasis is usually transmitted by contamination of drinking water and foods with feces (Oral-faecal route).
- Infection can be transmitted through autoinfection (by anal-oral contact).

Pathology

Symptoms appear after about 1 to 4 weeks later but sometimes more quickly or more slowly.

Asymptomatic amboebiasis

About 90% of cases are asymptomatic. The infected individual is still a carrier, able to spread the parasite to others through poor hygienic practices.
In asymptomatic infections the amoeba lives by eating and digesting bacteria and food particles in the gut.
It does not usually come in contact with the intestine itself due to the protective layer of mucus that lines the gut.
Cysts are found in the stool and can be present in an infected person for several years.

Amoebic dysentery

Disease occurs when amoeba comes in contact with the cells lining the intestine. It then secretes enzymes that destroy cell membranes and proteins.
Amoebic dysentery or ***amoebic colitis*** is the severe form of amboeiasis and it is generally known as invasive amoebiasis.

Symptoms

Amebic dysentery usually starts slowly over several days with abdominal cramps, and occasional loose stools, but progresses to diarrhea with blood and mucus.
Blood, mucus and pieces of necrotic tissue become more evident as the number of stools increases (10-20 or more per day) and stools will often contain little fecal material.
A few patients may develop fever, vomiting, abdominal tenderness, weight loss, or dehydration (especially children) as the severity of the disease increases.

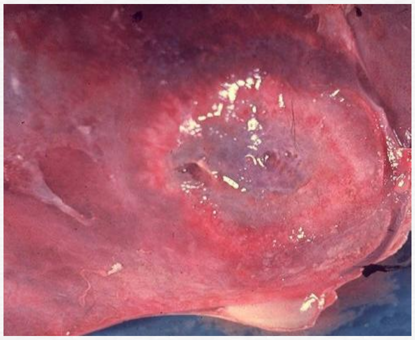
> **Note:** *Bacillary dysentery is a bacterial infection caused by Salmonellosis and Shigellosis, in this case No faecal matter are found and there are plenty of pus*

Complication of amboebiasis:

Intestinal complications

Severe ulceration of the gastrointestinal mucosal surfaces occurs in less than 16% of cases leading to rectal hemorrhage.
The most affected region of the large intestine is cacum where trophozoite invade mucosa and multiply in submucosa forming abcesses which breaks to form ulcers that is called ***bouton de chemise ulcers*** (flask shaped ulcer with narrow lumen and wide base).
Invasion of appendix that leads to clinical picture of appendicitis (surgery in this case may leads to peritonitis)
Repeated severe attacks may leads to amoeboma which is an inflammatory thickening of the intestinal wall around the ulcer which can be confused with cancer

Amoebic liver abscess



In fewer cases, (In about 10% of invasive cases) the amoebae enter the bloodstream and the parasite invades the soft tissues, most commonly the liver causing abscesses.

Amoebic hepatitis is ***Characterized by:*** Fever, nausea, vomiting, weight loss, right abdominal pain, and hepatomegaly

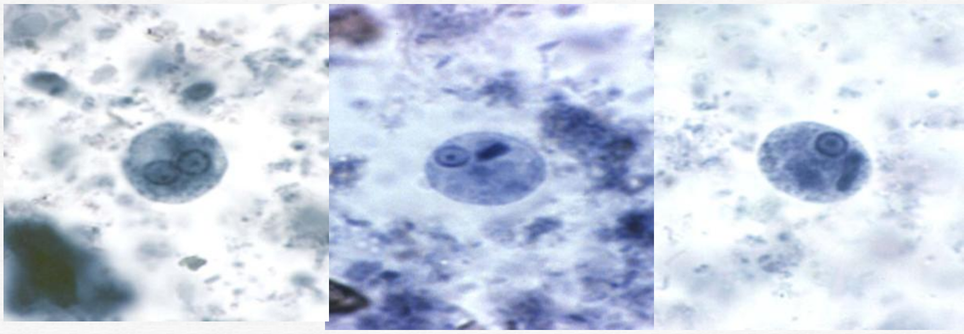
Diagnosis

1. Stool analysis

Asymptomatic infections

Are usually diagnosed by finding cysts shed in the stool

> **Note:** *Since cysts are not shed constantly, a minimum of three stools should be examined.*



Symptomatic infections

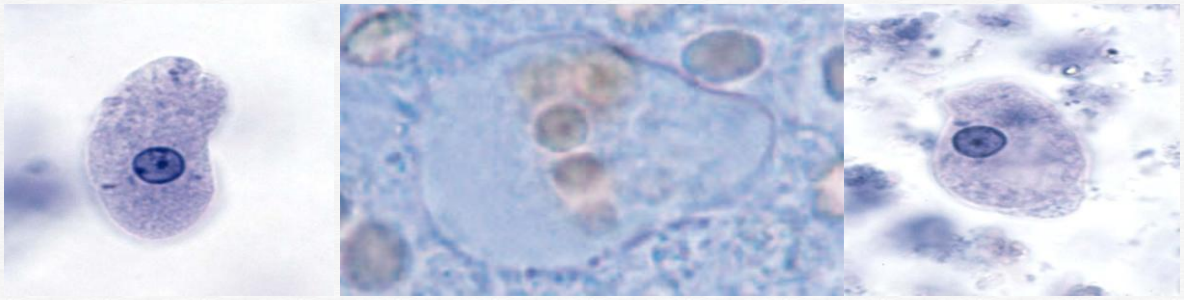
Gross examination

In the case of acute infections gross examination of stool show blood and mucus

Microscopic examination:

The motile form (trophozoite) can be seen in fresh feces. There are abundant RBCs with low WBCs.

> **Note:** *anti-diarrhea drugs should not be taken until stool examination is done*



Disadvantage of stool analysis:

One problem is that other parasites and cells can look very similar to *E. histolytica* when seen under a microscope.

For example ***Entamoeba histolytica*** and another ameba, called ***Entamoeba dispar***, which is about 10 times more common, look the same when seen under a microscope (morphologically identical) and should therefore be reported as E. histolytica/E. dispar.

Recommendation for lab

If you can not distinguish between E.histolytica and another ameba, it usually is best to assume that the parasite is *E. histolytica*.

Recommendation for doctor

The WHO recommends that infections diagnosed by microscopy alone should not be treated if they are asymptomatic.

2. Serology

Methods: Enzyme immunoassay (ELISA) or Indirect Hemagglutination (IHA)

There is an antibody response after invasive infection (liver abscess or colitis)

Most individuals will test positive for the presence of antibodies in serum only in cases of invasive amaebiasis.

When antibodies titer starts to rise? It takes 10-14 days to rise.

How long antibodies lasts in the body? Usually IgM disappear 6 months after treatment but IgG can persists up to 10 years.

> **Note:** *if the antibody test is negative but amoebic liver abscess is suspected a second sample should be done 7-10 days later and if negative also exclude amaebiasis.*

Clinical Implications

Titers <1:32 generally exclude amebiasis.

Positive test (titer > 1:128) indicates active or recent infection.

Titers range from 1:256 to 1:2048 may indicate Amebic liver abscess.

Prevention

- Wash hands thoroughly with soap and hot running water for at least 10 seconds after using the toilet or changing a baby's diaper, and before handling food.
- Clean bathrooms , toilet seats and taps.
- Avoid raw vegetables and fresh fruit when in endemic areas.
- Boil water or treat with iodine tablets.
- Do not eat or drink milk, cheese, or dairy products that may not have been pasteurized.
- Do not eat or drink anything sold by street vendors.

Giardia lamblia

Mode of infection

usually transmitted by contamination of drinking water and foods with feces (Oral-faecal route)

Infection can be transmitted through autoinfection (by anal-oral contact)

> **Note:** *Giardiasis does not spread via the bloodstream, nor does it spread to other parts of the gastro-intestinal tract, but remains confined to the lumen of the small intestine*

Pathology

Light infection

In humans, infection is Asymptomatic in about 70% of the patients.

Heavy infection

Only about 30% of patients exhibit symptoms, in this case Symptoms typically begin 1–2 weeks after infection and it includes:

- Fatty explosive diarrhea [***Steatorrhea***]
- Loose or watery stool with foul smelling
- Epigastric pain

- Stomach cramps
- malabsorption
- Loss of body weight
- Excessive gas (often flatulence or a foul or sulphuric-tasting belch, which has been known to be so nauseating in taste)
- An oily anal leakage or some level of fecal incontinence may occur

Complications of Giardiasis

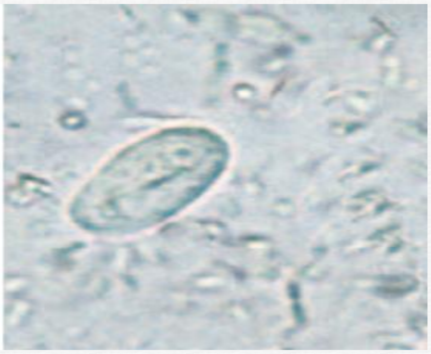
Lactase deficiency may develop in an infection with Giardia, however this usually does not persist for more than a few weeks, and a full recovery occurs

Some studies have shown that giardiasis should be considered as a cause of Vitamin B12 deficiency, this a result of the problems caused within the intestinal absorption system.

Diagnosis

Stool analysis

Asymptomatic infections

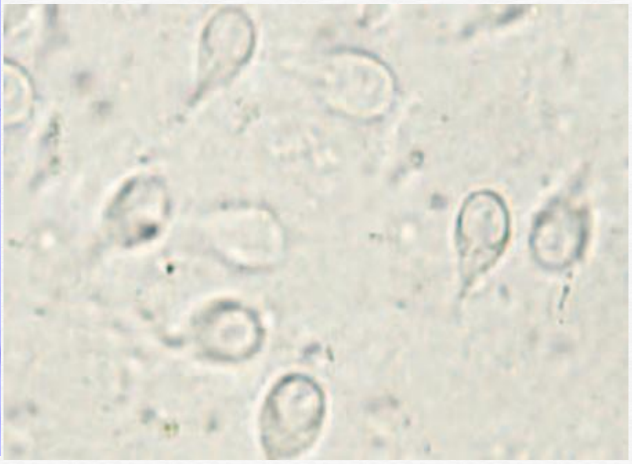


Finding G. lamblia cysts in more formed specimens. The cysts are excreted irregularly. Often large number may be present for a few days followed by fewer numbers for a week or more. Several specimens may need to be examined and a concentration technique used.

Symptomatic infections

The motile form (trophozoite) can be seen in fresh liquid feces.

Finding G. lamblia trophozoites in fresh diarrhoeic specimens particularly in mucus. They are often difficult to detect because they attach themselves to the wall of the intestine. Several specimens collected at different times may need to be examined.



Disadvantage of stool test:

Since the cysts and trophozoites are not shed consistently so many false negatives are found, some patients should be treated on the basis of empirical evidence; treating based on symptoms.

Fasciola hepatica

Synonymous Large liver flukes = common liver fluke or sheep liver fluke

Geographical distribution

F. hepatica has a cosmopolitan distribution it is found in areas where sheep and cattle are raised, and where humans consume raw watercress, including Europe, the Middle East, and Asia.

> **Note:** ***F. gigantica*** *is the species that is more common in tropics such as Africa, the Middle East*

Mode of infection

- Mammals acquire the infection by eating vegetation containing metacercariae after 12 hours encystation.
- Humans can become infected by ingesting metacercariae-containing freshwater plants, especially watercress.
- cercariae also encyst on water surface, humans can be infected by drinking of fresh untreated water containing metacercariae
- An experimental study suggested that humans consuming raw liver dishes from fresh livers infected with juvenile flukes could become infected.

Pathology

This may be divided into two major forms

1-Acute Fascioliasis

Is more commonly seen in sheep, and is not usually a feature of fascioliasis in man.

It occurs when very large numbers of metacercaria (>10 000) have been ingested at once. In these cases as the resulting large numbers of migrating larvae invade the liver they cause a traumatic hepatitis (liver rot), frequently resulting in death.

In some cases the liver capsule may rupture into the peritoneal cavity causing death due to peritonitis.

2- Chronic Fascioliasis

This form of the disease is much more common in all hosts, particularly man.

The development of infection in definitive host is divided into phases:

Incubation phase:

It takes few days to 3 months from the ingestion of metacercariae to the appearance of the first symptoms; depends on number of ingested metacercariae and immune status of host

Invasive or migratory phase

It is the fluke migration up to the bile ducts.

This phase is a result of mechanical destruction of the hepatic tissue and the peritoneum by migrating juvenile flukes.

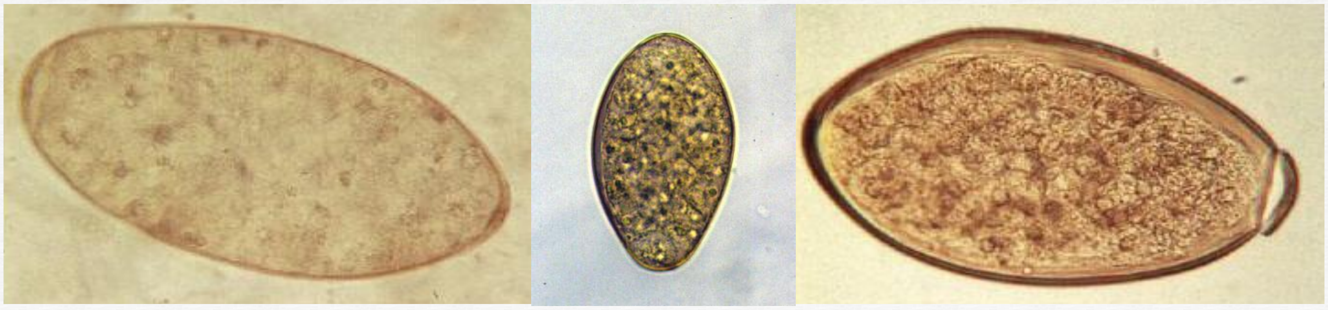
The major symptoms of this phase are:

- Intermittent fever: usually the first symptom of the disease; 40-42°C
- Abdominal pain pain under the right costal margin with hepatomegaly and splenomegaly
- Gastrointestinal disturbances: loss of appetite, flatulence, nausea, diarrhoea and fatty food intolerance
- Adult flukes in the bile ducts cause inflammation and hyperplasia of the epithelium
- Mechanical obstruction of the biliary duct that cause Jaundice, cholangitis and cholecystitis

Diagnosis

Stool analysis

Stool examination for detection of eggs.[the egg is characterized by its large size and opercalated shell. Usually appears 2 monthes after infection.



Disadvantage of the test:

Examinations of stool alone are generally not adequate because infected humans have important clinical presentations long before eggs are found in the stools. Moreover, in many human infections, the fluke eggs are often not found in the faeces, even after multiple faecal examinations.

Pseudofasciolosis

False fasciolosis refers to the presence of eggs in the stool resulting not from an actual infection but from recent ingestion of infected livers containing eggs. This situation (with its potential for misdiagnosis) can be avoided by having the patient follow a liver-free diet several days before a repeat stool examination.

If eggs are still present by repeated stool examination , the patient is really infected

If eggs disappear by repeated stool examination, the patient is not infected

Serological test

Antibody detection tests using ELISA or IHA are useful especially in the early invasive stages, when the eggs are not yet apparent in the stools, or in ectopic fascioliasis.

> **Note:** *Antibodies appear within 2 to 4 weeks after infection (5–7 weeks before eggs appear in stool).*

Advantage of the test:

Immunodiagnostic tests may be useful for early indication of Fasciola infection as well as for confirmation of chronic fascioliasis when egg production is low or and for ruling out "pseudofascioliasis" associated with ingestion of parasite eggs in sheep liver.

Antibody levels decrease to normal 6 to 12 months after chemotherapeutic cure and can be used to predict the success of therapy.

Disadvantage of the test:

Antibodies by ELISA have high sensitivity but may show cross-reaction with schistosomiasis and trichinosis.

> **Note:** *Biochemical and hematological examinations of human sera support the exact diagnosis (eosinophilia and elevation of liver enzymes).*

Hymenolepis nana

Synonymous Dwarf tapeworm

Mode of infection

- One can get infected by accidentally ingesting tapeworm eggs. This can happen by ingesting fecally contaminated foods and water, by touching your mouth with contaminated fingers, or by ingesting contaminated soil.
- The disease can be spread directly from person to person by eggs in feces via the fecal-oral route.
- Autoinfection : Eggs are infectious meaning they can re-infect you
- Internal autoinfection: An alternate mode of infection consists of internal autoinfection, where the eggs release their embryo, which penetrates the villus continuing the infective cycle without passage through the external environment

Pathology

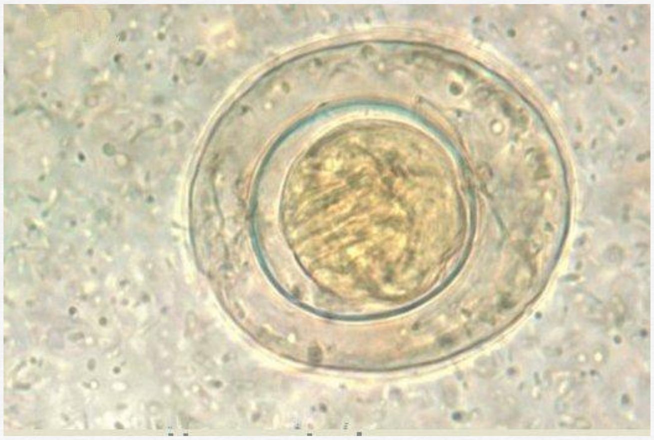
Light infection

Infection with the dwarf tapeworm is generally not serious. Most people who are infected do not have any symptoms.

Heavy infection

Those who have heavy infection may experience:
Nausea, weakness, loss of appetite (anorexia), diarrhea, and abdominal pain
Young children, may develop, itching around the anus or have difficulty sleeping

Note: *Contrary to popular belief, a tapeworm infection does not generally cause weight loss.*



Diagnosis

Examination of the stool for eggs and parasites Proglottids confirms the diagnosis

Note: *Identifying the parasites to the species level (H. nana or H. diminuta) is often unnecessary from a medical perspective, as the treatment is the same for both.*

Strongyloides stercoralis

Synonymous Dwarf thread worm

Mode of infection

- You can become infected by direct contact with contaminated soil, through Skin penetration.
- translactational transmission of infection(Transmammary infection)
- infection may occurs through organ transplantation.
- Autoinfection larva penetrate the wall of the lower ileum or colon external through perianal skin penetration .

Note: *Once a host is infected with S. stercoralis, infection is life-long unless effective treatment eliminates all adult parasites and migrating auto-infective larvae*

Pathology

Many people infected are usually asymptomatic at first.

Heavy infection passes in 4 stages:

1-Skin penetration stage

Dermatologic manifestations include swelling, itching (also called ground itch or dew itch), urticarial rashes and mild hemorrhage at the site where the skin has been penetrated and usually persists for 1-2 weeks.

> **Note:**: *Intense scratching may lead to a secondary bacterial infection*

2-Migratory stage

larva pass to the lung leads to:

Loffler`s syndrome

Can occur during pulmonary migration of the filariform larvae

Loeffler's syndrome is a disease in which eosinophil accumulates in the lung in response to a parasitic infection.

Eosinophil enzymes cause destruction of lung tissue leading to:

Dry cough chest pain, wheezing, haemoptysis and fever will sometimes be experienced by people who have been exposed to very large numbers of larvae

> **Note:**: *the disease is caused by the parasites Ascaris lumbricoides, Strongyloides stercoralis and the hookworms Ancylostoma duodenale and Necator americanus.*

> **Note:**: *Unlike that in ascariasis, pulmonary symptoms are uncommon and usually mild, except in severe infections.*

In heavy infection, larva passes to venous circulation then to the heart then go to different body organs causing: Visceral larva migrains

3-Intestinal stage

It is characterized by abdominal pain, nausea, anorexia, indigestion and diarrhea that usually develop around one month after infection.

4-Chronic stage

Infection last for many years due to autoinfection and this stage is characterized by: staetorrhea , weight loss and malabsorption syndrome

Complications

Persistent infection may leads to inflammation of mucosa and necrosis

Strongyloidiasis resulting from persistent infection can greatly mimic peptic ulcer and gallbladder disease. Many individuals with persistent strongyloidiasis undergo treatment or surgery for both peptic ulcer and gallbladder disease and then they fail to respond to the surgery or treatment.

> **Note:** *Giving proton pump inhibitors greatly reduces the HCl content of the stomach and allows the strongyloides to thrive. That is why often individuals with strongyloidiasis develop worse epigastric distress when placed on PPI's.*

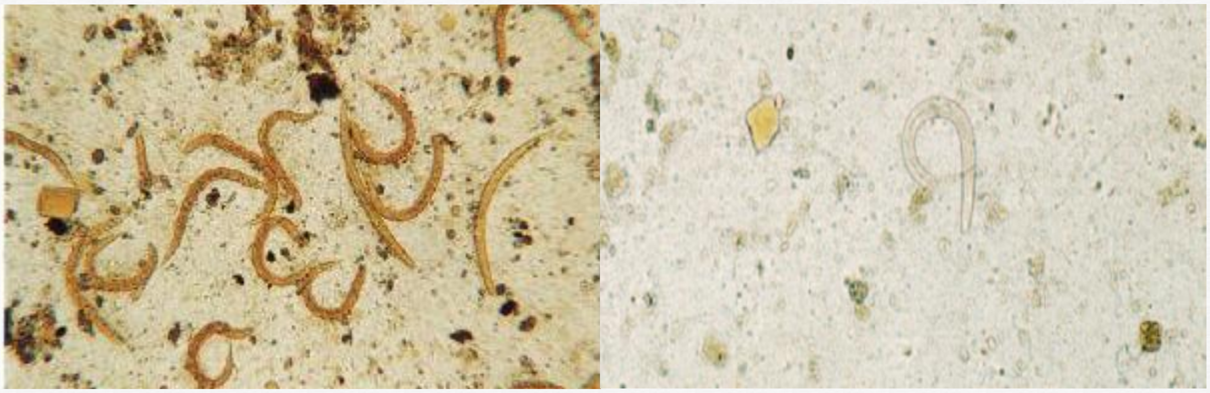
In more advanced cases strongyloidiasis can create nodulation in the lymphatic system of the small bowel. This can mimic Crohn's disease.

> **Note:** *It is important not to undergo steroid treatment for Crohn's if strongyloides is suspected. Doing so can lower one's immune system and then create immunocomprimization and can lead to a disseminated infection.*

Diagnosis

Stool analysis

It can be diagnosed by detection of the juvenile larvae in stool microscopically (appear 5 weeks after infectio)



larvae charachteristics

- it is actively motile
- it is a large larva measuring about 250 mm
- it show a typical large bulbed oesphagus

Note: *Finding strongyloides in the stool is negative in up to 70% of tests. It is important to undergo frequent stool sampling as well as duodenal biopsy if a bad infection is suspected.*

Note: *Direct examination must be done on stool that is freshly collected and not allowed to cool down, because hookworm eggs hatch on cooling and the larvae are very difficult to distinguish from strongyloides.*

CBC count

Leukocytosis is common.

Increase in eosinophils may also be present; indicates hyperinfection but the number usually decreases with chronicity;

Note: *Leukopenia and absence of eosinophilia are poor prognostic signs.*

Ancylostoma duodenale

Synonymous Old world hookworm

Mode of infection

- You can become infected by direct contact with contaminated soil, through Skin penetration.
- Infection occurs by accidentally swallowing contaminated soil.
- Translactational transmission of infection

  In a pregnant woman, after childbirth some or all of these larvae are stimulated to re-enter the circulation (presumably by sudden hormonal changes), then to pass into the mammary glands, so that the newborn baby can receive a large dose of infective larvae through its mother's milk.
- Transplacental from mother to the fetus

Note: *Hookworm cannot be spread person to person.*

Pathology

Infection passes in 4 stages:

1-Skin penetration stage

as Strongyloides

2-Migratory stage

as Strongyloides

3-Intestinal stage

as Strongyloides

4-Chronic stage

- This stage depend on number of worms and duration of infection
- The most significant risk of hookworm infection is anemia (Iron deficiency anemia), secondary to loss of iron (and protein) in the gut. The worms suck blood voraciously and damage the mucosa. However, the blood loss in the stools is occult blood loss

Note:: *hemoglobin decrease s proportional to the degree of infection*

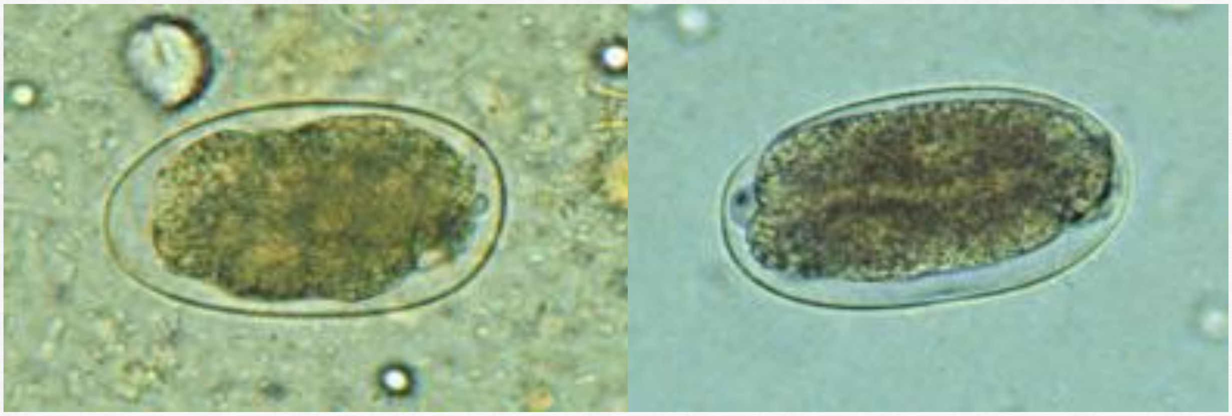
- Effects of anemia include: weakness, palpation, Pallor, chlorosis (greenish yellow skin discoloration), tachycardia
- Edema is caused by protein deficiency.
- Continuously infected children will have deficits in physical and intellectual growth, which may be irreversible.

Hookworms may cause, intrauterine growth retardation, prematurity, and low birth weight among newborns born to infected mothers.

Diagnosis

Stool analysis

It can be diagnosed by detection of the egg in stool microscopically (eggs appear 2 months after infection)



Note: *If the fecal sample is left for a day or more under tropical conditions, the larvae will have hatched out, so eggs might no longer be evident. In such a case, it is essential to distinguish hookworms from Strongyloides larvae, as infection with the latter has more serious implications and requires different management.*

CBC count

WBC is normal or slightly increased, with 15% to 30% eosinophilia begins at 4 weeks; peaks at 5 to 9 weeks when ≤75% eosinophils are present.

Cases of hookworm disease exhibit characteristic blood indices of iron deficiency anemia (eg, hypochromic microcytic).

Note: *When anemia is more severe, eosinophilia is less prominent*.

Ascaris lumbricoides

Synonymous Giant intestinal worm = round worm

Mode of infection

- Ingestion of Embryonated egg through contaminated food or water
- The source of transmission is from soil and vegetation on which fecal matter containing eggs has been deposited.
- Intimate contact with pets which have been in contact with contaminated soil may result in infection

Note: *Transmission from human to human by direct contact is impossible.*

Pathology

Infections are usually asymptomatic, especially if the number of worms is small.

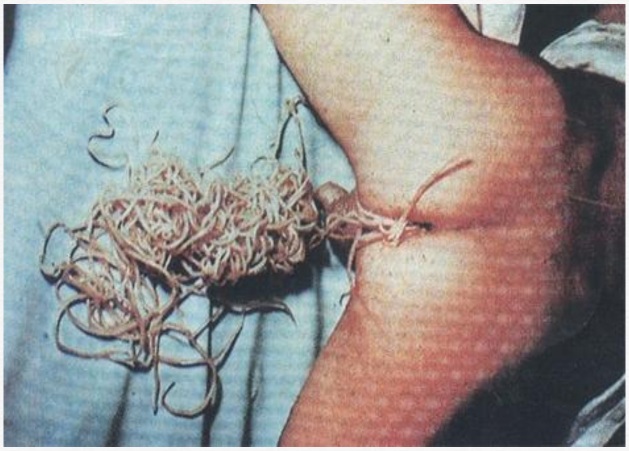
Generally the disease passes in 2 stages:

1-Migratory stage

- larva pass to the lung leads to ***loffler`s syndrome***
- In heavy infection, larva passes to venous circulation then to the heart then go to different body organs causing ***visceral larva migrains***

2-Intestinal stage

In this stage Ascariasis symptoms include:



- Nutritional deficiency (Ascaris takes most of its nutrients from the partially digested host food in the intestine)
- Indigestion and malabsorption due to production of anti enzymes interfere with digestion (specially protein indigestion)

Note:: *Ascaris infection does not produce the anemia associated with some other roundworm infections.*

Complications

Heavy worm infection may cause intestinal obstruction as :

- Appendicular obstruction leading to appendicitis
- Bile duct obstruction leading to jaundice
- Pancreatic duct obstruction leading to pancreatitis
- Intestinal wall perforation leading to peritonitis

In rare cases some worm may ascend to pharynx and either :

- Pass to larynx leading to suffocation
- Pass to middle ear leading to destruction
- Come out of mouth, nose.

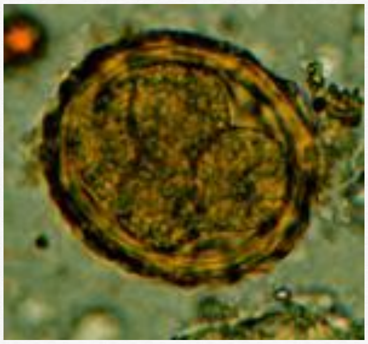
Ascaris-mediated anaphylactic shock syndrome

- This is a case of sudden death which my due to that ascaris worms release toxic substances causing mast cell de-granulation in all body tissues

Diagnosis

Stool analysis

Stool examination for detection of eggs (The characteristic feature of the egg is the coarse mammilated coating).



Note: *Blood counts may demonstrate peripheral eosinophilia during symptomatic phase.*

Note: *Occasionally, adult worms are spontaneously passed in stool.*

Enterobius vermicularis

Synonymous oxyuris = pin worm = seat worm = threadworm

Mode of infection

- Ingestion of egg via the fecal-oral route
- External auto-infection occurs by transferring infective eggs to the mouth with hands that have scratched the perianal area
- Retroinfection, or the migration of newly hatched larvae from the anal skin back into the rectum, may occur
- Person-to-person transmission can also occur through handling of contaminated clothes or bed linens.
- Some eggs may become airborne and inhaled. These would be swallowed and follow the same development as ingested eggs.

Pathology

Most symptoms of pinworm infection are mild; many infected people have no symptoms.
Except for itching [***Pruritus ani or itchy assis*** due to migration of female to peri-anal region] pinworm infestation does not usually cause any damage to the body.

Symptoms of heavy infection

- May include
- Painful itching around the anus
- Restless sleep [insomnia]
- Poor appetite
- Failure to gain weight

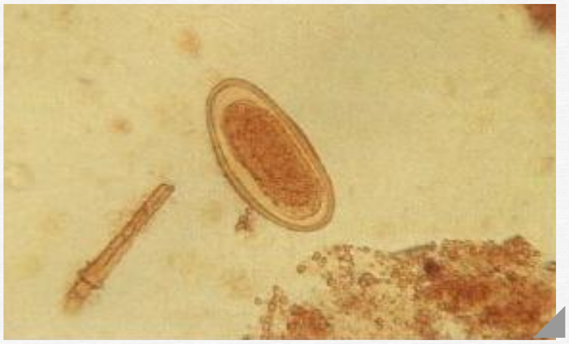
Complications

- ***Appendicitis***: 30% of cases are due to severe entrobiasis.
- Passage of worm to vagina or fallopian tube lead to ***vaginitis*** and ***salpingitis***

Diagnosis

Clinical diagnosis

Diagnosis is often made clinically by observing the female worm (or many worms) in the peri-anal region



Stool analysis

It can be diagnosed by detection of the larvated egg in stool microscopically

Note: *Morning sample is the best*

Trichostrangylus colubriformis

Synonymous The hairworm or bankrupt or black scour worm

Mode of infection
Ingestion of filariform larva via the fecal-oral route through contaminated food or H2O

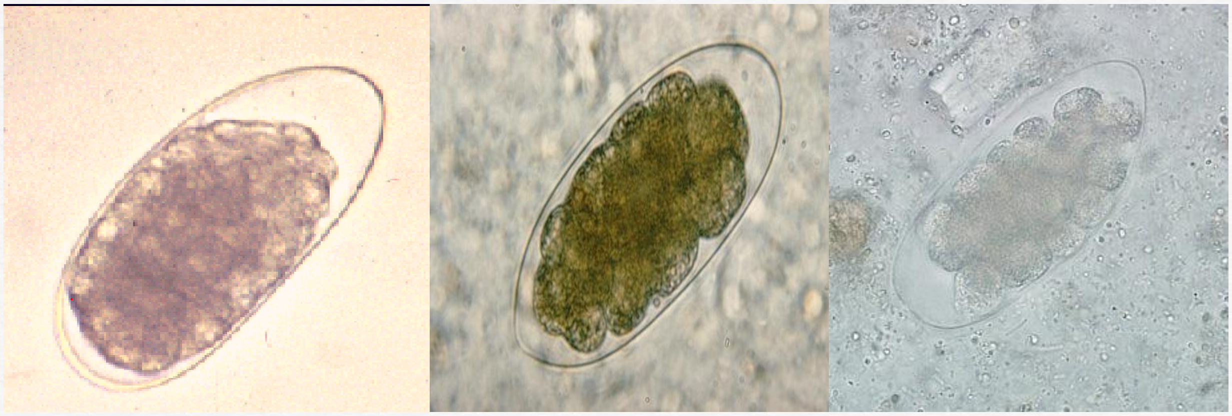
Pathology
Light infection
- Most people who are infected do not have any symptoms [asymptomatic infection].

Heavy infection
- Those who have heavy infection may experience:
- Mild abdominal discomfort, diarrhea, lack of appetite and weight loss
- Trichostongylus burrow into the mucosa and suck blood causing anemia

Diagnosis
Stool analysis
Examination of the stool for eggs confirms the diagnosis

> **Note:** *trichostrongyle eggs can be difficult to differentiate from each other and from hookworm eggs (but the eggs of the latter are smaller).*

> **Note:** *WBC and eosinophils Increased when patient is symptomatic.*



Trichuris trichiura

Synonymous *trichocephalus trichuris* = whip worm

Mode of infection
- The main risk factor for infection is ingestion of eggs from soil contaminated with feces.
- Ingestion of contaminated vegetables
- Accidental ingestion of eggs which are usually found in various grains

Pathology
Light infection
- Most people who are infected do not have any symptoms.

Heavy infection
- Those who have heavy infection may experience:
- Nausea, bloody diarrhea, and abdominal pain

- Heavy infection is characterized by profuse mucus with blood in the stool

Complications

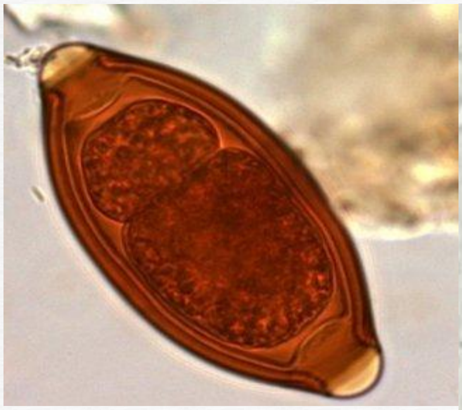
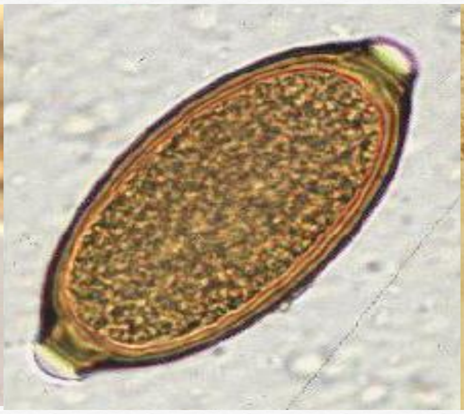
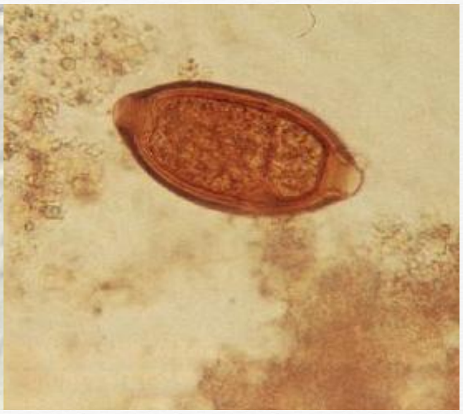
- Long-standing blood loss may lead to iron-deficiency anemia [hypochromic anemia].
- Toxic secretions of the worms cause hyperchromic anemia known as ***trichocephalic pernicious anemia***
- Rectal prolapse is possible in severe cases due to weakness of vator ani muscle.

Diagnosis

Stool analysis

Examination of the stool for eggs confirms the diagnosis

> **Note:** *The Trichuris eggs are lemon or football shaped and has terminal plugs at both ends.*
>
> **Note:** *WBC and eosinophils Increased in Trichuriasis .*

Schistosome mansoni & haematobium

Synonymous

Bilharzias blood-flukes

Geographical distribution

Found in parts of South America and the Caribbean, Africa, and the Middle East;

Habitat

S. mansoni : Mesenteric venous plexuses and small branches of Portal veins

S. haematobium: Pelvic venous plexuses around the urinary bladder

Mode of infection

- Drinking H_2O with cercaria may lead to infection where cercaria can penetrate mucus membrane above level of stomach as it is killed by gastric acidity.
- During washing or playing in infected canals cercaria can penetrate skin

Note: *Human contact with water is thus necessary for infection by schistosomes.*

Pathology

Pathogenicity of S. mansoni

Schistomiasis can be divided into 4 phases:

1-Stage of invasion

Penetration of the infective stage to the skin cause ***cercarial dermatitis***

It is also known as *Swimmer's itch, duck itch*, *Bather`s itch* or *Clam Diggers' Itch*

It is an immune reaction occurring in the skin of humans that have been infected by cercaria

It is commonly occur within hours of infection and do not generally last more than a week.

Symptoms Include itchy, raised papules, dermatitis

2-migratory phase

this phase lasting from penetration to maturity of worms

Katayama syndrome: develops a few weeks after 1st infection and characterized by allergic manifestations by metabolic products of worms ,Fever, cough, influenza-like symptoms, abdominal pain, eosinophilia, splenomegaly

3-acute phase (Stage of egg deposition)

occurs when the schistosomes begin producing eggs and lasts 3-4 months

the eggs pass through the intestinal wall and escape in faeces

this is accompanied by bleeding with dysentery in stool , abdominal tenderness weight loss and headache

4-chronic phase (Stage of tissue proliferation and fibrosis)

Occurs mainly in endemic areas and symptoms of this stage is different from tissue to other

Continuing infection may cause granulomatous reactions (bilharzioma or bilharzial granuloma) and fibrosis in the affected organs[eggs swims into circulation and reach many organs]

Granuloma formation is initiated by antigens secreted by the miracidium through microscopic pores within the rigid egg shell

Intestinal bilharzioma

Pathogenesis

When ova is trapped in the intestinal wall It lead to granuloma in the submucosa

Necrosis of mucosa cause ulcers and hemorrhage which lead to microcytic hypochromic anaemia

Sever prolonged infection cause ***sandy patches***, a lesion contain a large number of calcified ova healed by fibrosis

Hepatic bilharzioma

Pathogenesis

When ova reach the liver via portal vein branches it initiate acute inflammatory reaction that ends in chronic granuloma granulomas here giving rise to fibrosis of the liver which occur only many years after the infection

Fibrosis of the liver may be of two types:

Fine periportal fibrosis: if small number of eggs deposited and no cirrhosis occurs

Coarse periportal fibrosis (Symmers' fibrosis): if large number of eggs deposited

Complications

- Portal hypertention that cause Hepatosplenomegaly, esophageal varices , and pils
- Hypoalbuminaemia that leads to ascites
- Disordered liver functions

Pathogenicity of S. haematobium

Schistomiasis can be divided into 4 phases:

1-Stage of invasion

The same as S. mansoni

2-migratory phase

The same as S. mansoni

***3-acute phase* (Stage of egg deposition)**

occurs when the schistosomes begin producing eggs and lasts 3-4 months

egg pass through the bladder wall and appear in urine

this is accompanied by ***terminal haematuria*** , ***Polyuria*** (increased frequency of micturition due to inflammation) , ***Dysuria*** (burning pain during micturition)

***4-chronic phase* (Stage of tissue proliferation and fibrosis)**

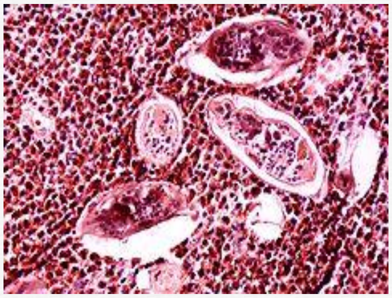
Occurs mainly in endemic areas and symptoms of this stage is different from tissue to other

Urinary bilharzioma

Pathogenesis

When ova is trapped in the urinary bladder wall It lead to granuloma

Prolonged and sever infection results in ulcers and sandy patches



Complications

microcytic hypochromic anaemia caused by prolonged hematuria

chronic bacterial infection of the bladder wall (Cystitis)

ova acts as a nucleus for phosphate stone formation

the infected bladder is suspected to the development of squamous cell carcinoma of the bladder

The bladder becomes calcified, and there is increased pressure on ureters and kidneys (hydronephrosis)

Diagnosis

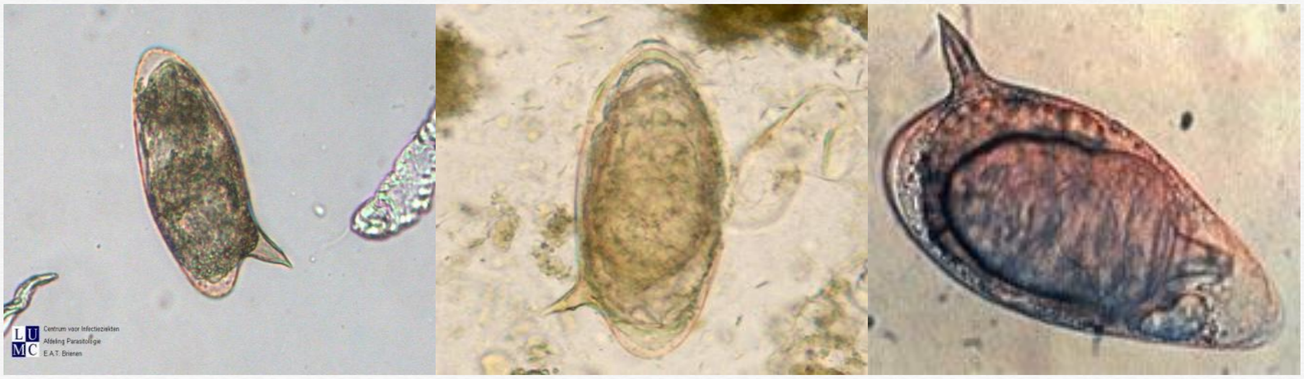
Diagnosis of S. mansoni

Stool analysis

It can be diagnosed by detection of the egg in stool microscopically

> **Note:** *Since eggs may be passed intermittently or in small amounts, their detection will be enhanced by repeated examinations and/or concentration procedures*

> **Note:** *Eggs of Schistosoma mansoni are approximately 140 by 60 µm in size and have a lateral spine.*



Serological test

- **Antibody detection**

Serology is initially still negative in early samples, but will become positive a couple of weeks later.

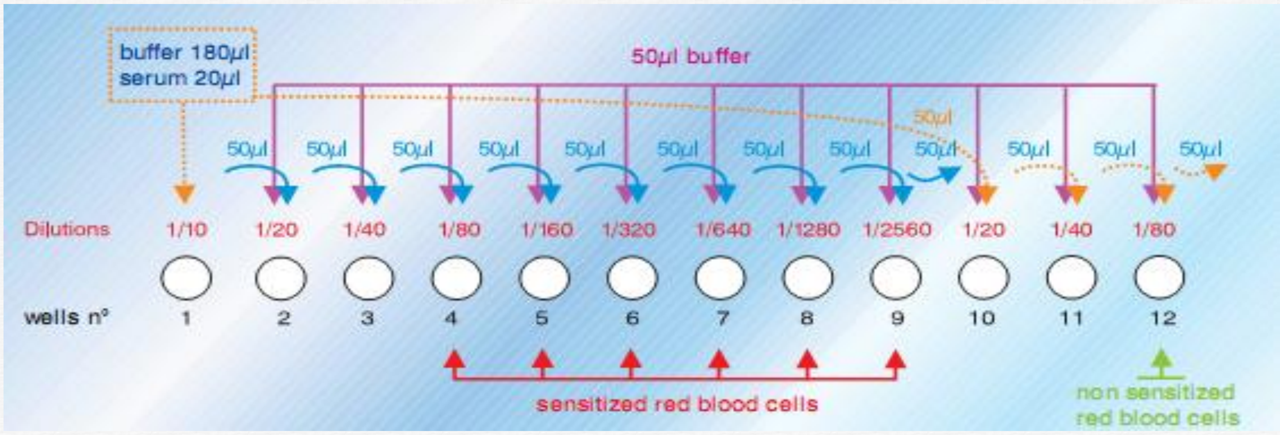
Procedure

Indirect hemagglutination (IHA) test (*Fumouze Diagnostics*)

The test using erythrocytes coated with Schistosoma mansoni adult worm antigens (WA)

Test steps

- Dilute serum an initial dilution1:20
- Use microplates
- Deliver 50 µl of buffer in wells n° 1 to 9
- Fifty microliters of a initial dilution of serum was added to well n° 1
- Mix together, then transfer 50 µl from the well n°1 into the well n°2, from the well n°2 into the well n°3, and so on until the well n°9 (serial dilutions).
- Deliver one drop of sensitized red blood cells into wells n°1 to 9
- Homogenize and wait for 2 hours.



Result

After incubation for 2 h at room temperature the titer in the test serum was recorded as one dilution before that which yielded a clear, sharp dark spot similar to those in the negative control wells.

Titers were expressed as reciprocal values.

The diagnostic titer is 1/160

High titre usually indicate recent infection

> **Note:** *Positive serological tests with low titres which cannot be confirmed parasitologically probably indicate either (1) an old, cured infection, (2) an infection with a very low worm load, (3) cross-reactivity with other worm species.*

Disadvantage

- The detection of anti-schistosomal antibodies is a useful tool for the diagnosis of schistosomiasis in patients who have recently visited an endemic area, but is of limited utility for the evaluation of patients indigenous to these areas
- It can not differentiate between active and post infection
- It can not differentiate between mild infections that are likely to remain asymptomatic from more severe infections
- Have a limited value in the follow up of many patients because the positive result remain too long after cure
- It may occasionally cross react with other helminthes

Antigen detection

Schisotosomes antigens

A variety of antigens secreted or excreted by the different life cycle stages of schisotosomes are present in the serum and/or the urine of the infected hosts. The antigens that are studied best for their diagnostic potential are the excretory-secretory antigens of adult worms also known as gut-associated antigens.

There are two proteoglycan gut-associated antigens appear promising for diagnosis of schistosomiasis. These are the ***circulating anodic antigen (CAA)*** also known as GASP (gut-associated schistosome proteoglycan) and the ***circulating cathodic antigen (CCA)*** also referred to as the M antigen.

Procedure

Enzyme-linked immunosorbent assay (ELISA)

ELISA has been developed for the detection of circulating schistosomal antigens in the sera and urine of actively infected patients with a limit of detectability of 0.5-5 ng antigen/ml.

Advantage

- The presence of these antigens in a patient indicates an active infection
- Positive results in individuals with negative egg counts indicate infections where adult worms are present but eggs are being produced at levels that cannot be detected in stool specimens.
- Antigen concentrations in serum and in urine are significantly correlated to the number of eggs excreted in stool or urine so it can be used to indicate the infection intensity.
- They also can be used to monitor the response to therapy, as Antigen levels in both serum and urine decline rapidly following successful chemotherapy

(Antigens was reported to disappear from 98% of sera and 97% of urines 12 weeks post-praziquantel treatment)

Diagnosis of S. haematobium

Clinical diagnosis

Clinical signs & symptoms of disease as :

- Haematuria is a common finding.
- Proteinuria is frequently present.
- Eosinophils can often be found in the urine.
- There is usually also a blood eosinophilia.

Urine examination

Examination of last drop of urine for detection of egg this help in acute cases when egg can be detected easily in urine .

Note: *Bacteriuria may accompany urinary schistosomiasis*

Note: *Terminal urine should be collected as the terminal drops contain a large proportion of the eggs.*

Toxoplasma gondii

Habitat Obligate intracellular parasite present in RBCs, muscles, viscera and brain

Disease Toxoplasmosis or Cat Scratch Fever

Reservoir Host Cats have been shown as a major reservoir of this infection.

Mode of infection

- Ingestion of raw or partly cooked meat, especially pork, lamb, or venison containing Toxoplasma ***cysts*** or ***pseudo cysts***
- Ingestion of contaminated food or water with the ***oocysts*** (occur through hand-to-mouth contact following gardening, or cleaning a cat's litter box)
- Transplacental infection in utero (congenital transmission)
- Receiving an infected blood transfusion

Pathology

Acute toxoplasmosis

It is characterized by:

- flu-like symptoms in the early acute stages
- swollen lymph nodes ,fever, and malaise
- muscle aches and pains that last for a month or more
- hepatosplenomegaly with slightly elevated liver enzymes
- CBC show atypical lymphocytosis, mild anemia, and leukopenia

Latent toxoplasmosis

- Most patients who become infected with Toxoplasma gondii and develop toxoplasmosis do not know it (there are no symptoms).

Toxoplasmosis and pregnancy

- If a woman receives her first exposure to toxoplasmosis while pregnant, the baby is at particular risk of transplacental transmission.
- If a woman infected before conception (6 months) do not transmit toxoplasmosis to the fetus unless the infection is reactivated during pregnancy by immunosuppression.

Note: *The presence of antibodies before pregnancy probably protects a fetus against toxoplasmosis at birth.*

Note: *For pregnant women with negative antibody titer, indicating no previous exposure to T. gondii, as frequent as monthly serology testing is advisable as treatment during pregnancy for those women exposed to T. gondii for the first time decreases dramatically the risk of passing the parasite to the fetus.*

Incidence of transplacental infection

- Infection in 1st trimester - is low incidence (15%)
- Infection in 2nd trimester -is moderate incidence (30%)
- Infection in 3rd trimester - is high incidence (65%)

If your fetus is infected during

- 1st trimester – abortion mostly occurs
- 2nd trimester - Congenital toxoplasmosis with severe symptoms
- 3rd trimester - Congenital toxoplasmosis with no or mild symptoms

Congenital toxoplasmosis

- It results from a primary (and often asymptomatic) infection acquired by the mother during pregnancy.
- Disease in the newborn may be severe, particularly if acquired early in pregnancy
- Most infants born to mothers infected during the 3rd trimester appear healthy at birth but are at high risk of mental retardation, Ocular toxoplasmosis, or other symptoms developing months or even years later (the second or third decade of life).

Symptoms

The classic tetrad signs suggestive of congenital toxoplasmosis include:

- bilateral chorioretinitis lead to ocular pain, blurred vision, and blindness
- hydrocephalus
- intracranial calcifications
- psychomotor retardation

Other possible symptoms of congenital toxoplasmosis include:

- hepatosplenomegaly
- Thrombocytopenia
- Jaundice
- Lymphadenopathy
- Fever
- Convulsions
- Deafness

Diagnosis

Who will undergo Toxoplasmosis infection diagnosis?

- Pregnant women with repeated abortion
- Pregnant women infected during pregnancy
- Baby of infected mother
- Patient with immunodeficiency disorder

Serological test

What are the serological tests used for diagnosis of toxoplasma?

IHA: for detection of total ***toxoplasma antibody***

A single positive serum sample proves only that the host has been infected at some time in the past. Serologic evidence for an acute acquired infection is obtained when antibody titers rise by a factor of 4 to 16 in serum taken 2 to 4 weeks after the initial serum collection

ELISA: for detection of ***toxoplasma IgM and toxoplasma IgG***

Results are usually given as positive or negative, indicating the presence or absence of IgG and IgM antibodies for each of the infectious agents

Note: *toxoplasma IgM appear during the first two weeks of illness peak within 4-8 weeks and then become undetectable with in 3-5 months*

Note: *toxoplasma IgG rise slowly, peak in 1-2 months and remain high for months or years*

What is the interpretation of serological tests of toxoplasma?

IgG	IgM	interpretation
-ve	- ve	May indicate that no infection is found ***Retest within 20-22 weeks of gestation and again near term***
+ ve or equivocal	+ ve	May indicate acute infection with in 12 months ***If patient is pregnant IgG avidity test should be done to exclude infection during past 3 months***
- ve	+ ve or equivocal	May indicate early acute infection (specimen was drawn very early after infection) or false +ve result ***Retest three weeks later*** - if the same result it most likely false +ve - if both become positive this suggest recent acute infection
+ ve	equivocal	May indicate infection with in 6 months ago ***Infection should be suspected in pregnant women*** By testing a second blood sample drawn two weeks later, the level of antibody can be compared. If the second blood draw shows an increase in IgG antibody, it may indicate a recent infection with the infectious agent.
+ ve	- ve	May indicate Infection more than 1 year ago ***There is protection now and in the future and maternal IgG protects the unborn baby***

Note: *For pregnant women with negative antibody titer, indicating no previous exposure to T. gondii, as frequent as monthly serology testing is advisable as treatment during pregnancy for those women exposed to T. gondii for the first time decreases dramatically the risk of passing the parasite to the fetus.*

Interpretation of results in congenital toxoplasmosis

- Presence of IgM antibodies in the newborn indicates high likelihood of infection with that organism. IgM antibodies produced in the mother cannot cross the placenta so presence of this type of antibody strongly suggests an active infection in the infant.
- positive IgG and absence of IgM in neonatal period has no indication because IgG can cross the placenta from infected mother to her baby (maternal origin)
- Persistent IgG titer in infant after 1y indicate congenital infection therefore serological testing should be repeated until IgG turns negative, exclusion of congenital Toxoplasmosis cannot be done before that

Note: *It is important to know if the baby has been infected to start treatment as early as possible.*

***Serological tests* limitations**

- Negative test results may occur early in the course of infection.
- During reactivation, an IgM response may not occur, or be present at levels too low to be detected.
- Interpretation of positive results may be difficult if patients have received blood transfusion within the past three months.
- Serologic testing is not useful for confirming the diagnosis in immunocompromised patients (AIDS) where IgM antibodies are not present during reactivation, and IgG antibodies to *T. gondii* do not distinguish between latent and reactivated infection.
- Other antibodies, such as rheumatoid factor or antinuclear antibodies may also cause a false-positive result.

Prevention

- Proper washing of vegetables before eating.
- Proper cooking meat (heat will kill the parasites).
- Pregnant women should avoid contact with cats and birds.
- Eat only well-cooked or previously frozen meat. Avoid dried meats, such as beef jerky.
- Do not drink unpasteurised milk, or eat other unpasteurised dairy products and avoid eating raw eggs.

Treatment

The aim of all medical intervention is the prevention of Congenital Toxoplasmosis.
Primary prevention is information about the ways of infection to avoid ingestion or inhalation. This is important for all pregnant women who are "seronegative"
Secondary prevention is the detection of infected women during pregnancy to start treatment before the fetus gets infected.
Tertiary prevention is the treatment of infected children to reduce or avoid symptoms.

Who must be treated?

Normally patient with healthy immunity has no serious health problems and need no treatment.
Treatment is often only recommended for:

- Patient with weak immune system (immunodeficiency disorder)
- Any infected pregnant women should be treated until delivery

> **Note:** *although a pregnant woman who has a healthy immune system is not personally at risk when infected, treatment is needed to protect her fetus.*

- The newborn baby (after diagnosis of a fetal infection or diagnosed at/after birth) should be treated

Medications that are prescribed for acute Toxoplasmosis are:

- ***Pyrimethamine antibiotic (daraprim)***
- ***Spiramycin antibiotic (Rovamycine)***

General bacteriology

What are Bacteria?

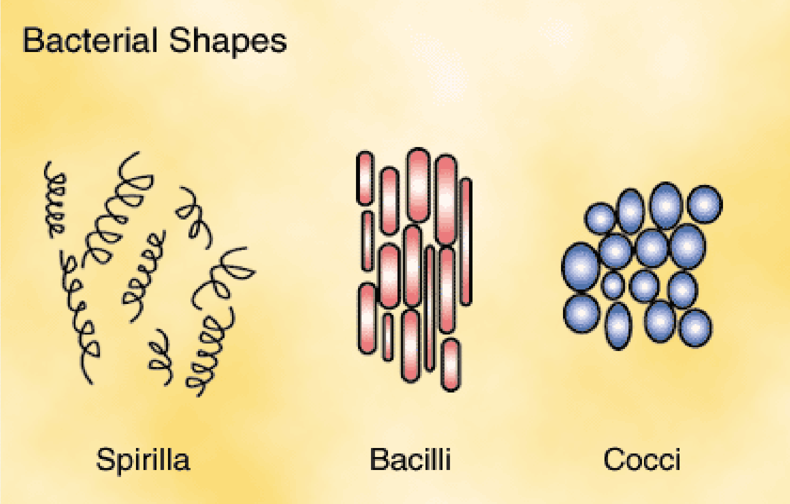
Bacteria are microscopic organisms (prokaryotic) whose single cells have neither a membrane-enclosed nucleus nor other membrane-enclosed organelles like mitochondria and chloroplasts.

> **Note** *Prokaryotic cells are not as complex as eukaryotic cells. They have no true nucleus as the DNA (a single chromosome) is not contained within a membrane or separated from the rest of the cell, but is coiled up in a region of the cytoplasm called the nucleoid.*

Classification of bacteria

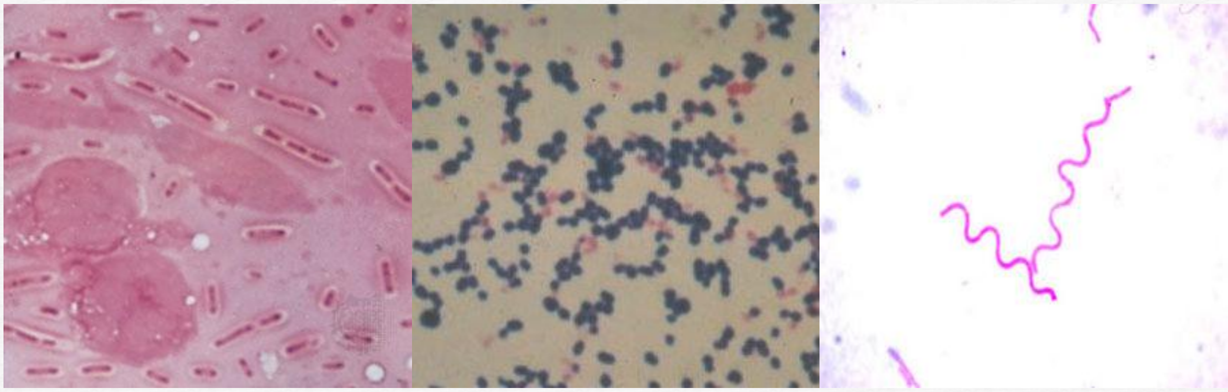
According to morphology

- **bacilli: rod-shaped**
- **cocci: spherical**
- **spirilla: curved walls**



According to energy production

- **Aerobic bacteria**
  It obtain its energy by oxidative process.
- **Anaerobic bacteria**
  Don't use oxygen and obtain their energy from fermentation reaction



Cell envelope

The cell envelope is the cell membrane and cell wall

Most bacterial cell envelopes fall into two major categories: ***Gram positive*** and ***Gram negative.***

Function of cell wall:

- Bacterial cell protection
- It give bacteria its shape

Gram stain:

It is a differential stain that is used to differentiate between two types of bacteria:

- **Gram-positive bacteria**

  It has the ability to retain a crystal violet-iodine stain when treated with alcohol.

- **Gram-negative bacteria**

  It does not retain a crystal violet-iodine stain after treatment with alcohol.

Gram-negative cell wall structure

It consists of two major segments:

A - Outer membrane (outer segment)

The outer membrane is a complex structure that is thought of as having two components:

- **peptidoglycan layer:**

  It is a polymer consisting of both sugar and peptide units.

- **Outer layer(outer membrane)**

  It is a mass of closely associated -phospholipids between two layers of proteins.

  Interspersed in the outer protein layer are many units of lipopolysaccharides (LPs).

B - Inner membrane (Plasma membrane)

Structure

It is a lipid bilayer with embedded protein.

Function

- Active and passive transport
- Cell respiration
- Waste products excretion
- It contains the enzymes that are needed for DNA replication and cell wall synthesis.

Periplasmic space

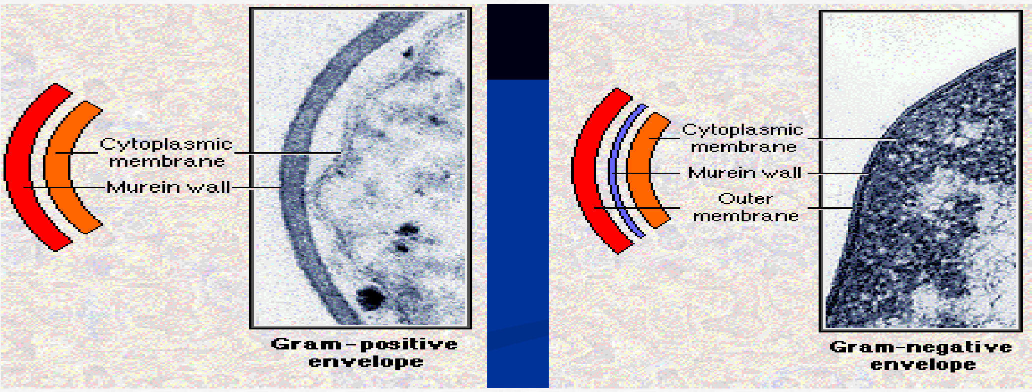
Between the peptidoglycan layer and the inner membrane is an aqueous region called the periplasmic space.

- It also contain portions that are needed for transport of substances across plasma membrane

Gram-positive cell wall structure

It differs from Gram-negative cell wall in three points:

- It lack the outer membrane
- The peptidoglycan layer is more thick (this cause stain to be retained)
- There is no periplasmic space

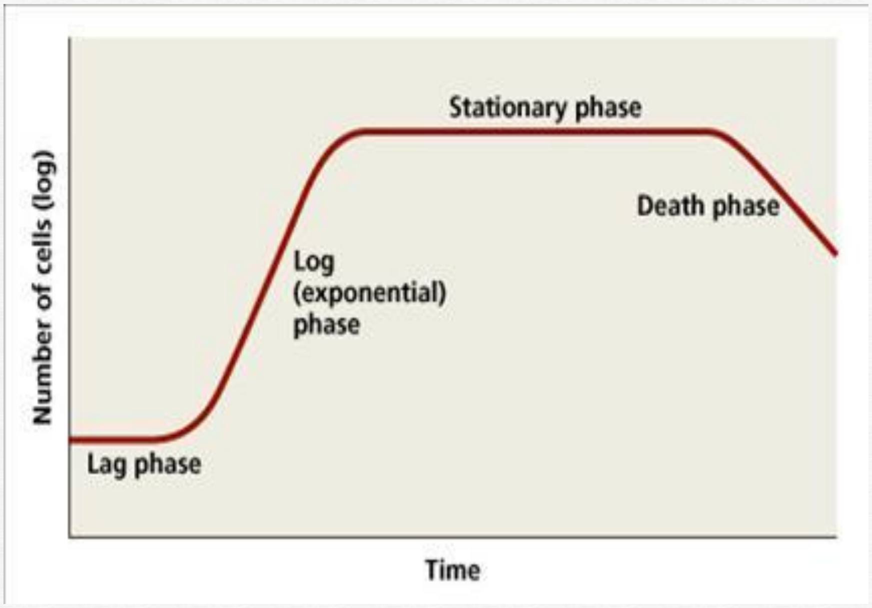


Bacterial growth cycle

When bacteria are inoculated in a medium, they slowly start to grow and divide.

The growth cycle of bacteria consists of four phases:

- ***Lag phase***
  No growth in this phase but the cell is metabolically active for synthesis of essential constituents of growth.
- ***Log phase (exponential phase)***
  In this phase bacterial cells starts to divide by binary fission. The bacteria will divide at a fixed time interval called the doubling time.



Note *Exponential growth: The number of cells per milliliter, the cell density, doubles repeatedly, giving rise to a logarithmic increase in cell number (2, 4, 8, 16,……)*

- ***Stationary phase***
  Growth continues until nutrients and O_2 become limiting and the growth rate decreases.
  So in this phase no further growth is possible and cell number becomes fixed.
- ***Death phase***
  Cells start to die after stationary phase if incubation continues due to:
  - Essential nutrients in the media are used up
  - Accumulation of waste products
  - Reduced O2 concentration owing to crowding

Pathogenic bacteria

If bacteria form a parasitic association with other organisms, they are classed as pathogens. Pathogenic bacteria are a major cause of human death and disease and cause infections such as tetanus, typhoid fever, diphtheria, syphilis, cholera, foodborne illness, leprosy and tuberculosis.

Two Mechanisms of pathogenic bacteria underlie the means by which they cause disease:

- **Invasiveness:**
  is the ability to invade tissues. It encompasses mechanisms for colonization (adherence and initial multiplication), production of extracellular substances which facilitate invasion and ability to bypass or overcome host defense mechanisms.

- **Toxigenesis:**
  is the ability to produce toxins. Bacteria may produce two types of toxins called exotoxins and endotoxins.

Salmonellosis (Widal test)

Salmonellosis is an infection with Salmonella bacteria.

- ***Enteric fever***

  Includes ***typhoid fever*** caused by Salmonella typhi and ***para typhoid fever*** caused by Salmonella para typhi A,B

Typhoid fever

Typhoid fever, is a common worldwide illness, transmitted by the ingestion of food or water contaminated with the feces of an infected person. The bacteria then perforate through the intestinal wall and are phagocytosed by macrophages.

- ***Causative agent***

It is caused by the bacterium ***Salmonella typhi***. The organism is a Gram-negative short bacillus that is motile due to its peritrichous flagella.

- ***Mode of infection***
  - Salmonella infections are zoonotic and can be transferred between humans and nonhuman animals.
  - Many infections are due to ingestion of contaminated food as meat, eggs and milk, as well as egg products.
  - Polluted surface water and standing water (such as in shower hoses or unused water dispensers)

    Note *Salmonella bacteria can survive several weeks in a dry environment and several months in water thus, they are frequently found in polluted water, contamination from the excrement of carrier animals being particularly important.*

  - A person may become an asymptomatic carrier of typhoid fever, suffering no symptoms, but capable of infecting others.

    Note *Approximately 5% of people who contract typhoid continue to carry the disease after they recover.*

- ***Signs and symptoms***

Classically, the course of untreated typhoid fever is divided into four individual stages, each lasting approximately one week.

In the first week:

- there is a slowly rising temperature with relative bradycardia, malaise, headache and cough.
- A bloody nose (epistaxis) is seen in a quarter of cases and abdominal pain is also possible.
- There is leukopenia, with relative lymphocytosis.

blood cultures are positive for Salmonella typhi or paratyphi. The classic Widal test is negative in the first week.

In the second week:

- the patient lies prostrate with high fever in plateau around 40 °C and bradycardia.

The major symptom of this fever is the fever usually rises in the afternoon up to the first and second week

- Rose spots appear on the lower chest and abdomen in around a third of patients.
- The abdomen is distended and painful in the right lower quadrant.
- Diarrhea can occur in this stage: six to eight stools in a day, green with a characteristic smell, comparable to pea soup.
- The spleen and liver are enlarged (hepatosplenomegaly) and tender, and there is elevation of liver transaminases.

The Widal reaction is strongly positive with antiO and antiH antibodies. Blood cultures are sometimes still positive at this stage.

In the third week:

a number of complications can occur:

- Intestinal hemorrhage due to bleeding in congested Peyer's patches; this can be very serious but is usually not fatal.
- Intestinal perforation in the distal ileum: this is a very serious complication and is frequently fatal.

It may occur without alarming symptoms until septicaemia or diffuse peritonitis sets in.

- By the end of third week the fever has started reducing (defervescence). This carries on into the fourth and final week.

- ***Treatment***

Typhoid fever in most cases is not fatal. Antibiotics, such as ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, Amoxicillin and ciprofloxacin, have been commonly used to treat typhoid fever.

Paratyphoid fever

Paratyphoid fevers are a group of enteric illnesses caused by strains of the bacterium ***Salmonella paratyphi***. There are three species of Salmonellae that cause paratyphoid: ***Salmonella paratyphi A, S. paratyphi B and S. paratyphi C***.

S. Paratyphi C has a limited distribution (common in Guyana and eastern Europe).

- They are transmitted by means of contaminated water or food.
- Disease caused by S. Paratyphi A and B is generally milder than typhoid. There is usually diarrhoea and vomiting and the entire intestinal tract may be inflamed, especially in B infections.

Diagnosis of Salmonellosis

Agglutination tests

When the antigen is particulate, the reaction of an antibody with the antigen can be detected by agglutination (clumping) of the antigen.

- **Direct agglutination**

  for example determination of blood types. Blood type can be determined by using antibodies that bind to the A or B blood group in a sample of blood.

- **Indirect agglutination (Passive hemagglutination)**
  - ***Passive haemoagglutination tests:***

    In case where RBSc are coated with known Ags and used for detection of specific Ab, these cases are known as Passive haemoagglutination tests. Used in diagnosis of Toxoplasma and Shistosoma.

  - ***Latex fixation test***

    In a latex fixation test, a sample is mixed with latex beads coated with antibodies. If the antigen is present, it will react with the antibodies causing the latex beads to clump. it is used in as ASOT , RF , CRP, widal and brucella.

Widal test

The ***Widal test*** is a presumptive serological test for enteric fever (latex agglutination test.) In case of Salmonella infections, it is a demonstration of agglutinating antibodies against antigens O-somatic and H-flagellar in the blood.

The important antigens used in the test are:

- Ag which is common for both Salmonella typhi and para typhi.
- Salmonella typhi H Ag for Salmonella typhi.
- Salmonella A for Salmonella para typhi A.
- Salmonella B for Salmonella para typhi B.

Antibodies appears in patient serum starting from 7th to 10th day of illness (O antibody appears after 7 days while H antibody appears after 10-12 days)

With treatment O antibody titre decrease while H antibody remain for long period

Positive O antibody indicate recent infection while positive H antibody indicate old infection

Procedure:

- The reagents must have the room temp. before using.
- Prepare white slide for using.
- Put 80 µ of the reagent A , B , O & H on the white slide separating each other by enough space then put 200 µ serum at each reagent zone
- Mix each reagent with it's neighbor serum quickly begin rotate the 4 reagents together for 1 min.

Reagent	Serum	Titre
80 ul	80 ul	1/20
80 ul	40 ul	1/40
80 ul	20 ul	1/80
80 ul	10 ul	1/160
80 ul	5 ul	1/320
80 ul	80 ul	1/640

- If no agglutination so the test is ***negative*** and the titer is 1/40 or 1/20.
- If agglutination occurs so the test is ***positive*** and repeat by dilution the serum with saline (serial dilution) and the titer is 1/80 , 1/160 , 1/320 , 1/640

Results

a single elevated titer >1:40 to the somatic (O) antigen or >1:80 to the flagellar (H) antigen] is suggestive only in unvaccinated individuals from non-endemic areas and in children <10 years of age from endemic areas. In endemic areas the Widal test is positive if O antigen titer is more than 1:80.

False positive results:

- In endemic areas the Widal test produces many false positive because S. Typhi shares O and
- H antigens with other enterobacteriacae and cross reactions occur ..
- False positive Widal test results are also known to occur in chronic liver disease associated with raised globulin levels and disorders such as rheumatoid arthritis, and nephritic syndrome.

False negative results:

- Too early collection of blood sample
- False negative Widal tests may be due to antibody responses being blocked by early antimicrobial treatment.
- Severe hypoproteinaemia may also prevent a rise in O and H antibody titres.

5-10% patients do not respond by antibody formation

Brucella test

Causative agent

Brucella sp. which is non-motile, non-encapsulated gram-negative bacilli.

Species

- *Brucella abortus* which infects cattle (the most common).
- *Brucella melitensis* which infects goats and sheep, (the most serious).

Members of this genus are pathogenic to animals from which they are transmitted to man causing brucellosis or undulant fever.

Habitat

small intestine

Disease

Brucellosis, also called **Malta fever**, or **undulant fever**

In cattle this disease is also known as **infectious abortion**.

> **Note:** *The popular name "undulant fever" originates from the characteristic undulance (or "wave-like" nature) of the fever which rises and falls over weeks in untreated patients.*

Mode of infection

- The most common mode in endemic areas, affecting farmers, and herdsmen is inhalation
- When sheep, goats, cows, or camels are infected, their milk is contaminated with the bacteria. If the milk is not pasteurized, these bacteria can be transmitted to persons who drink the milk or eat cheeses made it or if they eat raw liver.
- Skin (intact or broken) or mucus membrane contact: the bacteria enter the body through skin wounds.
- Direct person-to-person spread of brucellosis is extremely rare. Mothers who are breast-feeding may transmit the infection to their infants.

Pathogenicity

- Brucellae are intracellular organisms infecting reticuloendothelial cells of the spleen, liver, kidneys and bone marrow. From these sites, the bacteria pass into the blood.

The disease is characterized by acute undulating fever, headache, night sweats, fatigue and anorexia.

- Acute infection may resemble severe influenza with headache, sweating (especially at night) and generalized pains associated with fatigue and depression.
- Often the patient is anaemic and leukopenic with a relative lymphocytosis.

Prognosis

- Most patients recover completely without complications, if they receive appropriate antibiotic treatment.
- The relapse rate is approximately 10%, even with treatment.

Diagnosis

Brucella test

Antibody testing is the most reliable method for diagnosing brucellosis. Positive from the second week in titres of 1 : 80 reach maximum 3-6 weeks and disappears few months or years after recovery.

For brucellosis, only O-somatic antigen is used.

Procedure

The same as Widal test

- **Results**
  - A normal result shows no antibodies to Brucella.
  - As brucellosis progresses, more antibodies will be present and the titer 1/80 is diagnostic.
  - During and following treatment, antibody reactivity decreases. After 6 months, antibody levels are low or undetectable.

- **False negative result:** due to Prozone phenomenon or Pre-serological phase

Pre-serological phase:

During the first few days to weeks of exposure to an antigen, there may be very little antibody production. As brucellosis progresses, more antibodies will be present so if the doctor suspects brucellosis, he may need to have the test repeated 10 days to 2 weeks after the first test to watch for this rise.

Prozone phenomenon

The prozone effect is a lack of agglutination or clumping during tests for antibodies. It may result when the concentrations of antibodies are too high. During the prozone effect, extremely small antibody-antigen complexes fail to clump together and agglutinate visibly.
This means that despite negative readings, the test results are actually positive. While the prozone effect may have serious consequences concerning the results of blood serum testing, proper dilution of the antibodies can help prevent its likelihood.

- **False positive result**: due to cross reaction with E.coli

agglutination test and by the 2-mercaptoethanol (2ME) brucella agglutination test.

2-mercaptoethanol test

the use of 2-mercaptoethanol to separate the actions of specific IgG and IgM.
Often 2-mercaptoethanol is added. This agent more easily denatures the IgM class of antibodies, so if a decrease in the titer is seen after using this agent, it means that the contribution of IgM has been removed leaving the IgG component. This differentiation of antibody classes is important; as it allows for the distinction of a recent (IgM) from an old infection (IgG).

Brucella IgG and IgM

- Raised IgG antibodies indicate recent infection, raised IgM antibodies indicate active disease.
- The assay detects separately Brucella specific IgM and IgG antibodies. IgM antibodies develop early in brucellosis and remain present for several weeks to months following recovery. IgG antibodies develop later and can persist for months to years after recovery.

Streptococcus Sp. - Anti streptolysin O titer (ASOT)

Streptococcus is a genus of spherical Gram-positive bacteria

Streptococcal classification

As a rule, individual species of Streptococcus are classified based on their hemolytic properties. Alpha hemolysis is caused by a oxidation of iron in hemoglobin, giving it a greenish color on blood agar. Beta hemolysis is complete rupture of red blood cells, giving distinct, wide, clear areas around bacterial colonies on blood agar.

- ***Alpha-hemolytic***

  Streptococcus pneumoniae is the most common in this group. Despite the name, the organism causes many types of pneumococcal infection other than pneumonia, including acute sinusitis, otitis media, meningitis, bacteremia, sepsis, osteomyelitis, septic arthritis, endocarditis, pericarditis, and brain abscess.

- ***Beta-hemolytic***

  Group A

  For example ***Streptococcus pyogenes***, is the causative agent in Group A streptococcal infections, including streptococcal pharyngitis "strep throat" , acute rheumatic fever, scarlet fever, and acute glomerulonephritis.

  Group B

  For example ***Streptococcus agalactiae*** , causes pneumonia and meningitis in neonates and the elderly, with occasional systemic bacteremia.

Streptococcus pyogenes

Streptococcus pyogenes is a spherical gram-positive bacterium that grows in long chains and is the cause of Group A streptococcal infections. S. pyogenes displays streptococcal group A antigen on its cell wall. S. pyogenes typically produces large zones of beta-hemolysis (the complete disruption of erythrocytes and the release of hemoglobin) when cultured on blood agar plates)

- ***Pathogenesis***

S. pyogenes is the cause of many important human diseases ranging from mild superficial skin infections to life-threatening systemic diseases. Infections typically begin in the throat or skin. Examples of mild S. pyogenes infections include pharyngitis ("strep throat") and localized skin infection ("impetigo"). Infections due to certain strains of S. pyogenes can be associated with the release of bacterial toxins. Throat infections associated with release of certain toxins lead to scarlet fever.

- ***Complications***

Rheumatic fever and acute glomerulonephritis are abnormal immunological reaction to streptococcus, B-hemolyticus, group A infection.

Both conditions appear several weeks following the initial streptococcal infection. Rheumatic fever is characterised by inflammation of the joints and/or heart following an episode of Streptococcal pharyngitis. Acute glomerulonephritis, inflammation of the renal glomerulus, can follow Streptococcal pharyngitis or skin infection.

- ***Toxin***

Name	Description
Streptolysin O	An exotoxin that is one of the bases of the organism's beta-hemolytic property.
Streptolysin S	A cardiotoxic exotoxin that is another beta-hemolytic component. Streptolysin S is non-immunogenic . A potent cell poison affecting many types of cell including neutrophils, platelets, and sub-cellular organelles.

Streptococcal Tonsillitis

- ***What is streptococcal tonsillitis?***

A person with streptococcal tonsillitis has an inflammation of the tonsils, caused by an infection with the Streptococcus bacteria. The bacteria invade the tonsils in the back of the throat, resulting in inflammation and pain.

- ***What are the symptoms of streptococcal tonsillitis?***

The most common symptoms of streptococcal tonsillitis include a severe sore throat, throat redness, and swollen tonsils. Pus may be present on the surface of the tonsils. Additional symptoms of streptococcal tonsillitis include difficulty swallowing, headache, fatigue, fever, and swollen lymph nodes in the neck.

- ***Streptococcal Tonsillitis Risk Factors***
    - Air conditioning
    - Allergies
    - Chronic cough
    - Exposure to cold viruses
    - Living in a hot dry climate
    - Smoking or exposure to secondary smoke

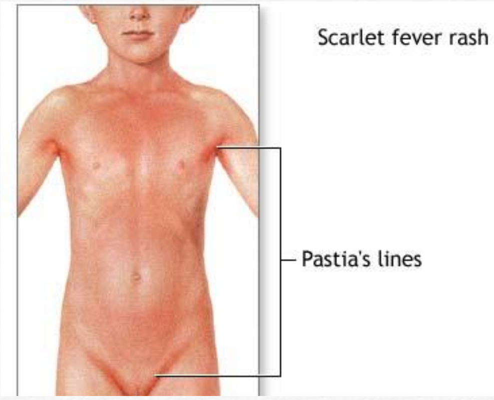
Scarlet fever

Scarlet fever is caused by an infection with group A streptococcus bacteria. The bacteria make a toxin that can cause the scarlet-colored rash from which this illness gets its name.

> **Note:** *Not all streptococci bacteria make this toxin and not all kids are sensitive to it. Two kids in the same family may both have strep infections, but one child (who is sensitive to the toxin) may develop the rash of scarlet fever while the other may not.*

- ***Symptoms of Scarlet fever***

The rash is the most striking sign of scarlet fever.
It usually begins looking like a bad sunburn with tiny bumps and it may itch.



The rash usually appears first on the neck and face, often leaving a clear unaffected area around the mouth. It spreads to the chest and back, then to the rest of the body.

Aside from the rash, there are usually other symptoms that help to confirm a diagnosis of scarlet fever, including:

- a reddened sore throat
- a fever above (38.3° Celsius)
- swollen glands in the neck
- The tonsils and back of the throat may be covered with a whitish coating, or appear red, swollen, and dotted with whitish or yellowish specks of pus.

When scarlet fever occurs because of a throat infection, the fever typically stops within 3 to 5 days, and the sore throat passes soon afterward. The scarlet fever rash usually fades on the sixth day after sore throat symptoms began, but skin that was covered by rash may begin to peel.

Rheumatic fever

Rheumatic fever is an autoimmune inflammatory disease which may develop after a Group A streptococcal infection (such as strep throat or scarlet fever) and can involve the heart, joints, skin, and brain.
Rheumatic fever primarily affects children between ages 6 and 15 years and occurs approximately 20 days after strep throat or scarlet fever.

Symptoms

In addition to fever, there are other main symptoms such as:

- **Arthritis**: is an inflammation of the joints that causes severe pain and swelling.
- **Rash**: a long lasting rash that begins on the trunk or arms as macules and spreads outward.
- **Carditis**: inflammation of the heart muscle which can manifest as congestive heart failure with shortness of breath, pericarditis, or heart murmur.
- **Sydenham's chorea** (abnormal movements) occurs in 30% of patients and is a movement disorder comprising of purposeless volatile movements of the face and arms.

Laboratory abnormalities

- increased Erythrocyte sedimentation rate
- increased C reactive protein
- leukocytosis
- rising Antistreptolysin O titre
- EKG changes (electrocardiogram)

Pathophysiology

Rheumatic fever is believed to be caused by antibody cross-reactivity. This cross-reactivity is a Type II hypersensitivity reaction and is termed molecular mimicry.

During Strep infection activated antigen presenting cells such as macrophages present the bacterial antigen to helper T cells. Helper T cells subsequently activate B cells and induce the production of antibodies against the cell wall of Streptococcus. However the antibodies may also react against the myocardium and joints, producing the symptoms of rheumatic fever.

Treatment

The management of acute rheumatic fever is geared toward the reduction of inflammation with anti-inflammatory medications such as aspirin or corticosteroids.
Individuals with positive cultures for strep throat should also be treated with antibiotics. Monthly injections of long acting Penicillin must be given for a period of 5 years in patients having one attack of Rheumatic fever but If there is evidence of carditis, the length of therapy may be up to 40 years.

Post-streptococcal glomerulonephritis

Post-streptococcal glomerulonephritis (PSGN) is an uncommon complication of either a strep throat or a streptococcal skin infection.

Symptoms

Symptoms of PSGN develop within 10 days following a strep throat or 3 weeks following a skin infection.
Symptoms include
Pallor due to edema and/or anemia, lethargy, loss of appetite, headache and dull back pain.

- ***Clinical findings***

  Hematuria

  May be microscopic and not identified by the patient.
  May be macroscopic and lead to dark brown or smoky urine.

  Oliguria

  Urine output is less than 400 ml/day (May be not observed by the patient)

  Edema

  Starts in the eyelids and face then the lower and upper limbs then generalized

  Hypertension

  It is usually mild to moderate.

- ***Pathophysiology***

The exact pathology remains unclear, but it is believed to be type III hypersensitivity reaction. Immune complexes (antigen-antibody complexes formed during an infection) become lodged in the glomerular basement membrane. Complement activation leads to destruction of the basement membrane.

- ***Laboratory abnormalities***
  - There will be elevated levels of ASOT
  - low complement levels (C3 and C4) in the blood.

Antistreptolysin-O (ASO):

This test measures the amount of antistreptolysin O (ASO) in the blood. ASO is an antibody targeted against streptolysin O, a toxin produced by Group A streptococcus bacteria.

- ***Why Get Tested?***
  - To help determine whether a person has had a recent Group A streptococcal infection
  - To help diagnose post-streptococcal sequelae of rheumatic fever and glomerulonephritis

- ***Procedure :***
  - Reagent must have the room temperature before use then mix gently
  - Put 20 µl of serum in a black slide then put on it 20 µl of the reagent .
  - Mix and rotate the slide by hands in a circle direction for 2 min .

If no precipitation occurs the result is negative.
If precipitation occurs the result is positive and give titre 200 IU / ml .

In positive case we must repeat by the following steps:

- Serum diluted by saline by the ratio 1 : 1 (make serial dilution)
- Repeat the test as above

If negative so the titre 200 IU /ml .
If positive the titre 400 IU /ml . and try another dilution. The Titer in this test 200 - 400 - 600 - 800 IU/ml

- ***Normal***

ASO titer: less than 200 IU/ml (Todd units)

- ***Interpretation***

The ASO test alone is positive in 80% to 85% of group A streptococcal infections

ASO antibodies are produced about a week to a month after an initial strep infection. ASO levels peak at about 4 to 6 weeks after the illness and then taper off but may remain at detectible levels for several months after the strep infection has resolved.

Note: *Rising titre is more significant in diagnosis than single test.*

Results:

- Results more than 200 is diagnostic.
- Results more than 400 is definitely elevated.

> **Note:** *ASOT production is especially high in rheumatic fever and glomerulonephritis (may reach 1000 IU/ml). These conditions show marked ASO titer increases during the symptomless period preceding an attack.*

Disadvantage

The ASO test does not predict if complications will occur following a streptococcal infection, nor do they predict the type or severity of the disease.

Interfering Factors

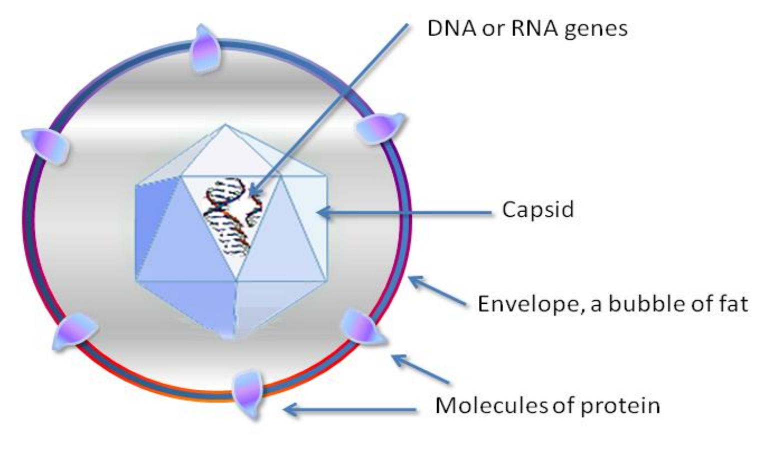
- Antibiotic therapy suppresses streptococcal antibody response.
- Early use of penicillin prevents rise of titers.
- False-positive results are associated with TB, liver disease (e.g., active viral hepatitis)
- Latex agglutination method may give a false-positive result in markedly lipemic specimens.

General virology

Virus structure

Virus is a small infectious agent (10-30 nm) in diameter. It is metabolically inert, since it cannot replicate outside of a living cell (it needs a living cell).

A complete virus particle, known as a virion, consists of nucleic acid surrounded by a protective coat of protein called a capsid. These are formed from identical protein subunits called capsomers. Viruses can have a lipid "envelope" derived from the host cell membrane.



Classification of virus

The Baltimore Classification of viruses is based on the method of viral mRNA synthesis Baltimore classification is a classification system that places viruses into one of seven groups depending on a combination of their nucleic acid (DNA or RNA), strandedness (single-stranded or double-stranded), Sense, and method of replication.

Viruses can be placed in one of the following groups:

DNA viruses:

- dsDNA viruses (e.g. Adenoviruses, Herpes viruses)
- ssDNA viruses DNA (e.g. Parvoviruses)

RNA viruses:

- dsRNA viruses (e.g. Reoviruses)
- ssRNA viruses (+)sense RNA (e.g. Picornaviruses, Togaviruses)
- ssRNA viruses (−)sense RNA (e.g. Orthomyxoviruses, Rhabdoviruses)
- ssRNA-RT viruses (+)sense (e.g. Retroviruses)

Mechanism of infection

There are six basic, overlapping stages in the life cycle of viruses in living cells:

- **Attachment** is the binding of the virus to specific molecules on the surface of the cell. This specificity restricts the virus to a very limited type of cell. For example, the human immunodeficiency virus (HIV) infects only human T cells.
- **Penetration** follows attachment; viruses penetrate the host cell by endocytosis or by fusion with the cell.
- **Uncoating** happens inside the cell when the viral capsid is removed and destroyed by viral enzymes or host enzymes, thereby exposing the viral nucleic acid.
- **Replication** of virus particles is the stage where a cell uses viral messenger RNA in its protein synthesis systems to produce viral proteins.
- **Assembly** takes place in the cell when the newly created viral proteins and nucleic acid combine to form hundreds of new virus particles.
- **Release** occurs when the new viruses escape or are released from the cell. Most viruses achieve this by making the cells burst, a process called lysis. Other viruses such as HIV are released more gently by a process called budding.

Hepatitis A virus

*Hepatitis A (formerly known as **infectious hepatitis**)* is an acute infectious disease of the liver caused by the hepatitis A virus (HAV)

Genome

The virus contain ssRNA genome

Incubation period

Incubation period 3-5 weeks (mean 28 days)

Mode of infection

- It is most commonly transmitted by the fecal-oral route via contaminated food or drinking water.
- Also infection is reported to occur by contaminated needle.

Pathogenicity

Virus enters via the gut; replicates in the alimentary tract and spreads to infect the liver, where it multiplies in hepatocytes.

Viraemia is transient. Virus is excreted in the stools for two weeks preceding the onset of symptoms.

Symptoms usually begin 2 to 6 weeks after infection.

In adults, early symptoms include:

- nausea (upset stomach)
- loss of appetite
- vomiting
- diarrhea
- loss of energy
- weight loss
- fever
- abdominal cramps (stomach or side pain).
- Itching
- jaundice may also be present but do not occur in all cases.

Some people with hepatitis A, especially young children, may have no symptoms or only a mild flu-like illness without jaundice

Prognosis

- Most people recover within 3 weeks.
- Unlike hepatitis B and C, hepatitis A infection does not become long term or chronic.
- Fulminant hepatitis is rare: 0.1% of cases (leading to liver failure)

Diagnosis

- **Liver function test:**

  It is done to support a diagnosis of hepatitis.

Bilirubin is eleviated in blood and urine.

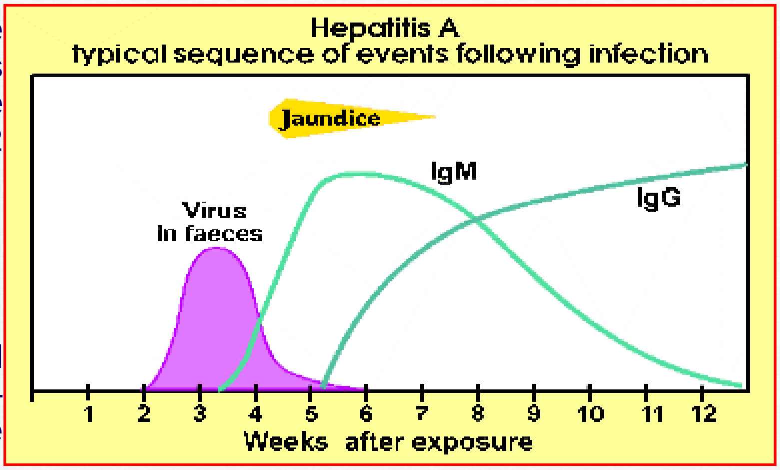
GOT and GPT are highly elevated (reach more than 1000 U/l)

Eleviatd GOT and GPT still elevated for 1-3 weeks

➤ **Serology:**

- **Anti-HAV antibodies (IgM type)**

IgM anti-HAV antibodies generally can be detected in the blood as early as 2 weeks after the initial HAV infection. These antibodies disappear from the blood 3 to 12 months after the infection.



IgM antibodies indicate a recent infection with hepatitis A virus.

- **Anti-HAV antibodies (IgG type)**

About 8 to 12 weeks after the initial infection with hepatitis A virus, IgG anti-HAV antibodies appear and remain in the blood for lifelong protection against HAV.

IgG anti-HAV antibodies mean that you have had a hepatitis A viral infection (Old infection).

Prevention

- Wash hands with soap and warm water before preparing or eating food.
- Keep bathrooms clean and disinfected after every use.
- Cook shellfish thoroughly before eating.
- Drink water from approved sources only.

Immunization

- ***Passive immunization:***
  Normal immunoglobulin given to travelers to third world countries
- ***Active immunization***
  inactivated cell culture vaccine

Treatment

There is no specific treatment for hepatitis A. Sufferers are advised to rest, avoid fatty foods and alcohol (these may be poorly tolerated for some additional months during the recovery phase and cause minor relapses), eat a well-balanced diet, and stay hydrated.

Hepatitis B virus

Hepatitis B (also known as "Dane particles" or Australia antigen)

Genome

It contain a circular dsDNA genome

HBV proteins

- **s =** surface (coat) protein
- **c =** inner core protein which surrounds the viral DNA (it is not found in blood)
- **e =** secreted protein with unknown function

Incubation period

Incubation period 2 - 5 months

Mode of infection

- ***Pparenterally transmitted:*** through blood transfusions, sharing of needles, tattooing, renal dialysis, and organ donation
- ***Sexually transmitted:*** through unprotected sexual contact
- ***Horizontal transmission:*** in children (children's institutions), families, 'close personal contact'.
- ***Vertical transmission:*** this means the transplacental transmission from carrier mother to baby through contact with maternal blood during delivery. (rare route)

A mother who is positive for HBsAg confers a 20% risk of passing the infection to her offspring at the time of birth.

Hepatitis B is rarely transmitted through breast milk

Pathogenicity

The virus replicates in the liver and virus particles, as well as excess viral surface protein, are shed in large amounts into the blood.

Viraemia is prolonged and the blood of infected individuals is highly infectious.

Symptoms usually begin 6 weeks to 6 months after exposure to the virus (average 2 months).

In adults, early symptoms include:

- nausea (upset stomach)
- loss of appetite
- vomiting
- abdominal cramps (stomach or side pain).
- jaundice also present.

Nearly all infants and children and 50% of adults with a new infection have no symptoms whatsoever.

Prognosis

- most people recover within 3 weeks.
- Over 90% of infants, 50% of children, and 5% of adults with acute hepatitis B will develop chronic or long-term infection.
- Fulminant hepatitis is rare; accounts for 1% of infections.

Chronic infection

infected individuals who fail to eliminate the virus completely become persistently infected. ***Those who are at particular risk include:*** babies, young children, immuno-compromised patients

The virus persists in the hepatocytes and on-going liver damage occurs because of the host immune response against the infected liver cells.

The chronic carrier still potentially infectious , although have no symptoms

Chronic infection may take one of two forms:

- Chronic persistent Hepatitis: the virus persists, but there is minimal liver damage and no complications
- Chronic Active Hepatitis: There is aggressive destruction of liver tissue and rapid progression to cirrhosis or liver failure.

Patients who become persistently infected are at risk of developing hepatocellular carcinoma

HBV is thought to play a role in the development of this malignancy because:

- 80% of patients with HCC are carriers of hepatitis B.
- Virus DNA can be identified in hepatocellular carcinoma cells.
- Virus DNA can integrate into the host chromosome.

Diagnosis

- **Liver function test:**
  - AST and ALT levels are eleviated moderately to 100-300 U/i
  - Albumin decreased in advanced cases

- **Serology:**

Viral antigens:

- **Hepatitis B surface antigen (HBsAg)**

is a protein antigen produced by HBV. This antigen is the earliest indicator of acute hepatitis B and frequently identifies infected people before symptoms appear.
HBsAg disappears from the blood after 3-6 months during the recovery period.

if it persists for more than 6 months it indicate a chronic infection (carrier state)

carrier state

Sometimes, HBV goes into “hiding” in the liver and does not produce new viruses that can infect others. People who have this form are said to be carriers. In other cases, the body continues to make viruses that can further infect the liver and can be spread to other people. In both these cases, HBsAg will be positive.

So how to differentiate between these two states?

- **Hepatitis B e-antigen (HBeAg)**

is a viral protein associated with HBV infections. Unlike the surface antigen, the e-antigen is found in the blood only when there are viruses also present. It is synthesized under condition of high viral replication.

It is considered as a sensitive index of viral replication.

Use of HBeAg

- When the virus goes into “hiding,” the e-antigen will no longer be present in the blood. HBeAg is often used as a marker of ability to spread the virus to other people (infectivity).
- Measurement of e-antigen may also be used to monitor the effectiveness of HBV treatment; successful treatment will usually eliminate HBeAg from the blood and lead to development of antibodies against e-antigen.

Its persistence for more than 10 weeks suggests the development of chronic state

Viral antibodies:

- **Hepatitis B surface antibody (Anti-HBs)**

- Surface antibody (anti-HBs) becomes detectable late in convalescence after disappearance of HBsAg, and indicates immunity following infection. it remained for life and is not found in chronic carrier.
- anti-HBs can also be acquired from successful vaccination.

- **Hepatitis B e antibody (Anti-HBe)**

- In those who have recovered from acute hepatitis B infection, anti-HBe will be present along with anti-HBc and anti-HBs.

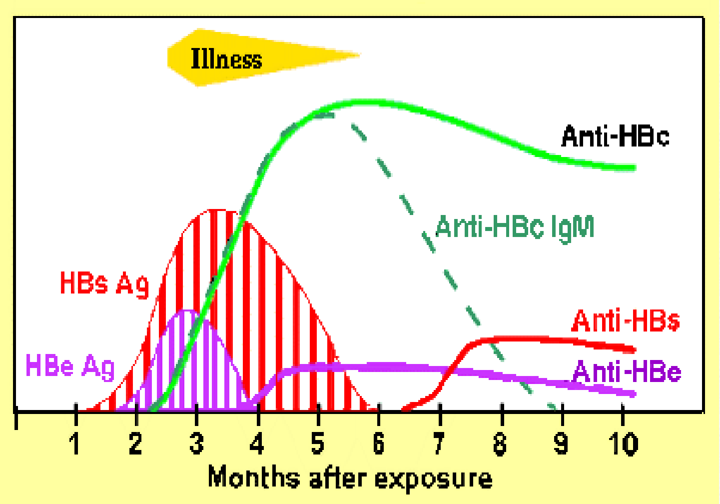
Its appearance is a strong evidence that the patient will recover completely.

- In those with chronic hepatitis B, usually anti-HBe becomes positive when the virus goes into hiding or is eliminated from the body.

becomes detectable as viral replication falls. It indicates low infectivity in a carrier.

❖ Hepatitis B core antibody (Anti-HBc)

The core antigen is found on virus particles but disappears early in the course of infection. HBcAb is produced during and after an acute HBV infection.



Anti-HBc is either

- Core IgM rises early in infection and indicates recent infection.
- Core IgG rises soon after IgM, and remains present for life in both chronic carriers as well as those who clear the infection.

Window phase

Some times HBsAg disappears several weeks , before HBsAb can be detected , this is called window phase in this case HBcAb is positive and used in diagnosis in this situation.

> **Note:** *The inability to detect HBsAg may result from it being complexed with HBsAb antibodies in the "window" phase*

> **Note:** *Positive HBcAb and Positive HBsAb indicates that the patient is immune to HBV as a result of having been infected previously (indicated by the presence of anti-HBc antibodies which would not occur if the patient had been vaccinated)*

> **Note:** *Negative HBcAb and Positive HBsAb indicates that the patient is immune because of vaccination against HBV.*

Immunization

- ***Active Immunization***

  Two types of active vaccines are available:

  - Serum derived prepared from HBsAg purified from the serum of HBV carriers
  - Recombinant HBsAg made by genetic engineering in yeasts

Both vaccines are equally safe and effective. The administration of three doses induces protective levels of antibodies in 95% of vaccine recipients.

- ***Passive Antibody***

Hepatitis B immune globulin should be administered to non immune individuals following single episode exposure to HBV-infected blood. For example: needle stick injuries.

Treatment

There are currently several treatments for chronic hepatitis B that can increase a person's chance of clearing the infection. Treatments are available in the form of antivirals such as lamivudine and adefovir and immune system modulators such as interferon alpha.

Hepatitis C virus

Hepatitis C used to be called non-A, non-B hepatitis.

Genome

It contain a ssRNA genome

Note: *virus genome have hyper variable regions , this property prevent vaccination*

HBV proteins

- **Structural proteins:** E (envelope) M (matrix) C (core)
- **Non-structural proteins:** NS1, NS2, NS3, NS4, NS5

Incubation period

Incubation period 6-8 weeks

Mode of infection

Anyone infected with hepatitis C can spread the infection, even if no symptoms are present or the liver tests are normal.

- ***parenterally transmitted:*** through blood transfusions, sharing of needles (Intravenous drug abusers), tattooing, renal dialysis, and organ donation

Blood exposure can also happen from sharing razors, toothbrushes, nail files, or clippers that an infected person has used.

- ***Sexually transmitted:*** Hepatitis C virus can be spread through sexual activity (in 1-3% of cases) but it is not common among people with one steady, long-term partner. People who have multiple sex partners have a greater risk of getting hepatitis C.
- ***Vertical transmission:*** About 5% of babies of infected mothers get hepatitis C either during pregnancy or at childbirth.

There is no evidence hepatitis C virus is transmitted through breastfeeding unless the mother's nipples are cracked or bleeding.

Pathogenicity

- After a person is infected with hepatitis C, it takes several weeks before the infection is established in the liver. This is called the incubation period.
- Most people (86%) with hepatitis C do not know that they are infected because they do not feel sick.
- Some people do get mild symptoms that are flu-like, such as nausea, fatigue (feeling tired), loss of appetite, fever, headaches, and abdominal pain.

Jaundice (yellow eyes or skin) may occur but is not common

- Fatigue may be the main symptom of chronic hepatitis C infection.

❖ Chronic infection

- HCV Causes a milder form of acute hepatitis than does hepatitis B but 50% individuals develop chronic infection, following exposure.
- The disease progress at slow rate in many patients during the first two or more decades after infection.
- The most common complications of chronic HCV are cirrhosis and Hepatocellular carcinoma
- One of the characteristic clinical feature of chronic HCV is the wide array of extra hepatic manifestations such as:
  - ✓ 60% of individuals with chronic HCV have auto antibodies as ANA, RF, ASMA
  - ✓ 20 % of individuals has immune disease such as autoimmune thyroditis

Diagnosis

➢ Liver function test:

Hepatitis C can cause abnormal liver function tests. Even people with no symptoms can have abnormal liver function tests.

- Elevated ALT often in a fluctuating pattern are the most characteristic feature

ALT is normal in about 30% of chronic HCV cases

- Persistent ALT is indication of active liver disease
- Albumin decreased in advanced cases

➢ Serology

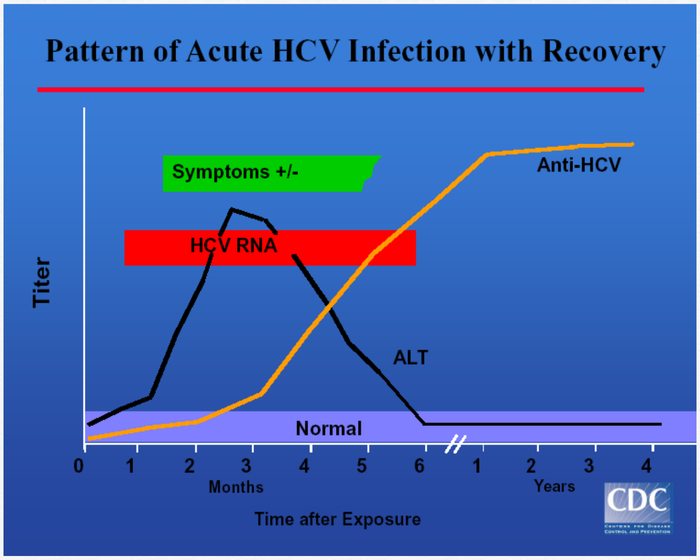
Anti-HCV antibody tests detect the presence of antibodies to HCV in the blood, indicating an HCV infection has occurred.

However, this test makes no distinction between an acute or chronic infection.

Note: *There is some evidence that, if your test is "weakly positive," it may not mean that you have been exposed to the HCV virus. It is recommended that weakly positive tests be confirmed with the next test before being reported.*

Types of HCV serological tests:

Rapid HCV chromatographic test device



It is a qualitative , membrane based immunoassay for the detection of antibody to HCV in serum, during testing the serum reacts with the recombinant HCV antigens coated particles , the mixture migrates upward on the membrane by capillary action to reacts with HCV antigens on the membrane and generate a colored line.

Anti-HCV Tri-Dot test

The test is based on the principle of inter-second antibody enzyme immunoassay for the determination of anti-HCV in serum using recombinant HCV antigens

HCV antigens are immobilized on a porous immunofiltration membrane , sample and reagents pass through the membrane and are absorbed into the underlying absorbent pad

As the patient's sample passes through the membrane , HCV antibodies if present in serum , bind to the immobilized antigens , in the subsequent washing step unbound serum proteins are removed.

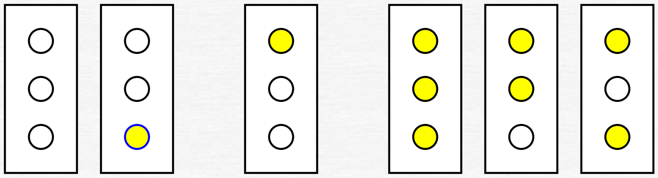
In the next step , the protein-A conjugate is added which binds to the Fc portion of the HCV antibodies to give distinct pinkish purple dot against a white background at the test region.

ImmunoComb test

The ImmunoComb test is a modified ELISA, which has been described as a "dot"-ELISA that detects antibody levels in serum or whole blood.

The test detects three HCV antigens (core antigen, NS3 , NS4 antigen) and this increase the sensitivity of the test.

Results



Invalid ***Negative*** ***Positive***

faintly colored lower spot must be suspected as +ve ====➔ must be retested
faintly colored middle spot must be suspected as –ve ====➔ must be retested

ELISA (Enzyme Linked ImmunoSorbent Assay)

Hepatitis C infection is usually diagnosed using the screening test called ELISA (Enzyme Linked ImmunoSorbent Assay), which tests for an antibody against hepatitis C.

HCV *PCR:*

HCV genetic material (RNA) testing uses polymerase chain reaction (PCR) to detect the RNA of an active hepatitis C infection. The RNA can be detected in a person's blood within 1 to 2 weeks after exposure to the virus.

Qualitative HCV tests

- It is done to confirm a positive result on an HCV antibody test.
- HCV antibodies from the mother may persist in infants until 15 months of age. If an early diagnosis is desired, testing for HCV RNA can be performed between the ages of 2 and 6 months

Qualitative HCV RNA is reported as a “positive” or “detected” if any HCV viral RNA is found; otherwise, the report will be “negative” or “not detected”.

Quantitative HCV test

- Viral Load or Quantitative HCV tests measure the number of viral RNA particles in blood.
- Viral load tests are often used before and during treatment to help determine response to treatment by comparing the amount of virus before and after treatment (usually after 3 months).

successful treatment causes a decrease of 99% or more in viral load soon after starting treatment (as early as 4-12 weeks), and usually leads to viral load being not detected.

Quantitative HCV RNA is reported as numbers are virus equivalents per Milliliter (level of viremia)

below 200,000 very low

200,000 to 1,000,000 low

1,000,000 to 5,000,000 medium

5,000,000 to 25,000,000 high

above 25,000,000 very high

Immunization

There is no vaccine for hepatitis C.

Public Health recommends people with hepatitis C be vaccinated against hepatitis A and B.

Interferon α treatment

Interferon is a anticancer drug used in the treatment of tumors, leukemia, and certain types of cancer and viral infections.

Recommendation:

Antiviral therapy is recommended for patients with greatest risk of progression to cirrhosis:

- Positive anti-HCV
- Persistently increased ALT
- Detectable HCV-RNA
- Liver biopsy showing at least moderate inflammation and necrosis or fibrosis

How to take interferon?

- Interferon is given by injection usually three to five times a week.
- Interferon can be taken alone or in combination with Ribavirin

Treatment with interferon alone is effective in about 10% to 20% of patients. Interferon combined with ribavirin is effective in about 30% to 50% of patients.

Precautions:

Interferon is NOT recommended for use during pregnancy or if your breast-feeding.

Side effects:

Interferon may cause flu-like symptoms such as headache, fever, vomiting, nausea, muscle aches and pains, fatigue, and chills. These symptoms may go away once your body adjusts to the Interferon.

Response to therapy:

This can be indicated as follow:

- 50% show normal serum ALT
- 33% lose detectable HCV-RNA in serum; is associated with remission.

Decreased interferon response occurs in <15% of patients indicated by higher HCV-RNA titers

Presence of HCV-RNA in serum after sustained response to interferon indicates late relapse. (50% relapse after therapy occurs)

Cultures & sensitivity

Overview

Culture is defined as a laboratory test by which samples from body specimens are cultivated in a special growth medium in order to isolate the microorganisms that may be present. Culture is a highly effective laboratory method for identifying the microorganisms that cause infectious disease and for obtaining a definitive diagnosis.

Why It Is Done

Generally culture and sensitivity test is done to:

- Detect and identify bacteria that may be causing an infection
- Identify the best antibiotic to treat the infection.

Culture samples

Specimen collection for culture and sensitivity testing must be carried out under standard precautionary techniques in order to avoid contamination of the specimen.

Urine sample

A urine culture may be done when an abnormal result from a urinalysis (such as an increased number of white blood cells) indicates an infection.

Sampling

- Patient must not take any antibiotics before testing by 48 hours.
- Specimens should be obtained in a sterile containers and must be of sufficient quantity
- morning mid-stream specimen of urine for quantitative bacteriuria examination is ideal specimen.
- The sample must be in the lab for testing within 30 minutes after taking.

Note: *Using diuretics or drinking a large amount of liquid. This may dilute your urine and lower the concentration of bacteria, causing inaccurate test results.*

Stool sample

A stool culture is done to detect and identify certain types of bacteria, that can cause symptoms of an intestinal disease which include prolonged diarrhea, bloody diarrhea, an increased amount of gas, abdominal pain and fever.

Note: *More than 50 different kinds of bacteria normally live in the intestines. However, disease can result if numbers of abnormal bacteria in the intestines increase..*

Sampling

- Patient must not take any antibiotics before testing by 48 hours.
- Specimens should be obtained in a sterile containers and must be of sufficient quantity
- The sample must be in the lab for testing within 30 minutes after taking.

Wound sample (Pus)

A skin or wound culture is a test to detect and identify bacteria that may be infecting the skin or a wounds such as injuries, burn, surgical wound or animal bites.

Note: *symptoms of an infection often include pain at the site, redness, tenderness, swelling, warmth, red streaks toward the body, swollen lymph nodes, and the presence of pus.*

Sampling

- Patient must not take any antibiotics before testing by 48 hours.
- Specimens should be obtained by a sterile swab and must be of sufficient quantity
- Pus sample must be taken from deep inside the wound

❖ Throat swab sample

A throat culture may be done to determine the cause of a sore throat. A throat culture helps to distinguish a bacterial infection from a viral infection. Identifying the organism that is causing the sore throat.

Sampling

- Place the patient's mouth in good visual light.
- Use a sterile throat culture swab.
- Tilt head back. Depress the patient's tongue with a tongue blade and visualize the throat as well as possible.
- Rotate the swab firmly and gently over the back of the throat, around both tonsils, and on areas of inflammation, exudation, or ulceration.

Notes:

- *Do not touching the tongue or lips with the swab to avoid contamination.*
- *Do not touch the posterior pharynx , other wise the patient will vomit*
- *Because most patients gag or cough, the collector should wear a facemask for protection.*
- *Refrigerate the throat culture if examination is delayed.*

❖ Blood sample

A blood culture can detect and identify bacterial infection in the blood. The blood does not normally contain any bacteria but bacteria can enter the bloodstream as a severe complication of infections (like pneumonia , osteomyelitis or meningitis), during surgery (especially when involving mucous membranes such as the gastrointestinal tract), or due to catheters and other foreign bodies entering the arteries or veins (including intravenous drug abuse). A bacterial infection is usually serious because the blood can spread the bacteria to any part of the body.

Note: *A blood culture is also done to evaluate unexplained fever , (**fever of unknown origin**)*

Note: ***Bacteremia** is a medical term referring the presence of bacteria in the blood while **Septicemia** is a medical term referring to the presence of pathogenic organisms in the blood-stream, leading to sepsis (in which multiplying bacteria release toxins into the blood stream and trigger the production of cytokines,causing fever, chills, toxicity, tissue anoxia, reduced blood pressure).*

Sampling

- Incubate media bottles before taking the sample at 37 degree for at least10 minutes
- But the label on the bottle indicating the patient identification
- Disinfect the skin carefully to avoid contamination with skin microorganisms.
- Blood is obtained by inserting a needle into the vein then draw about 10 ml of blood and put it into two culture bottles containing broth to grow aerobic and anaerobic microorganisms.
- Inoculate the culture bottles carefully and incubate the bottles in incubator

Note: *do not store inoculated bottles in the refrigerator*

Note: *Multiple samples (two or three blood samples from different veins) are usually taken to increase the chances of identifying bacteria in the blood and to have a better chance of ruling out a false positive blood culture (results from skin contaminant)*

Culture media

Types of culture media

- ***Nutrient agar***
  is a microbiological growth medium commonly used for the routine cultivation of non-fastidious bacteria.
  Agar is a polysaccharide formed of agarose and agaropectin , it is used to solidify culture media because:
  - It has a highly gelling capacity
  - Free of substances toxic to bacteria

  Note: *This medium is the base of different types of media*

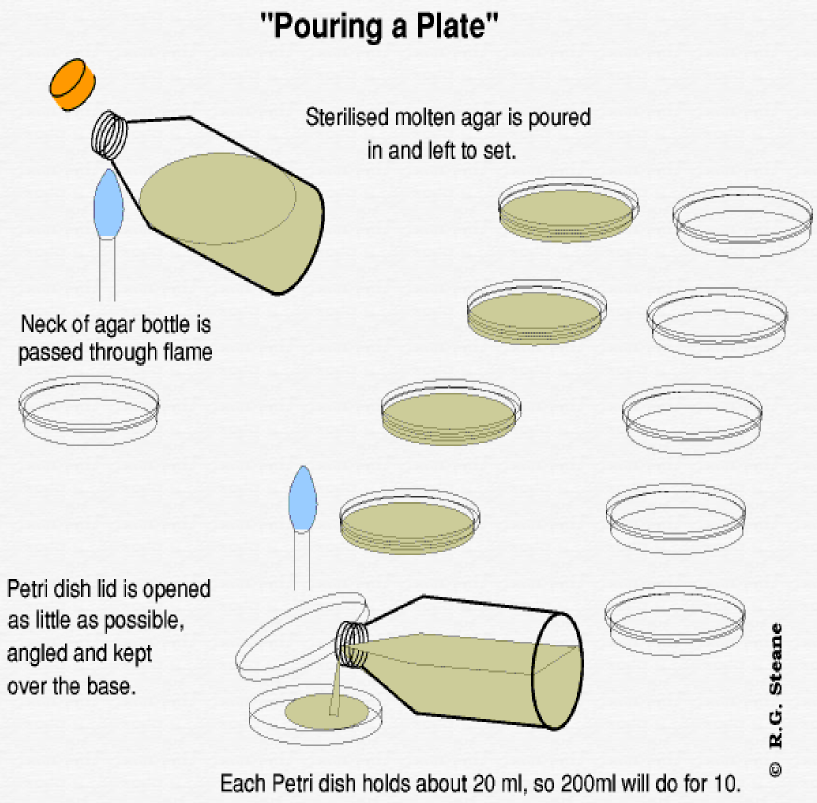
- ***Blood agar plate (BAP)***
  Contains mammalian blood (usually sheep or horse), typically at a concentration of 5–10%. BAP are an enriched, differential media used to isolate fastidious organisms and detect hemolytic activity.
- ***Chocolate agar (CHOC)***
  A type of blood agar plate in which the blood cells have been lysed by heating the cells to 56 °C. Chocolate agar is used for growing fastidious bacteria.
- ***MacConkey agar (MAC)***
  A selective and differential media used to differentiate between Gram negative bacteria while inhibiting the growth of Gram positive bacteria.
- ***Salmonella Shigella Agar (S.S Agar)***
  is a selective growth medium used in the isolation of Salmonella and Shigella species from clinical samples

Preparation of growth media

- Weight an accurate amount of solid agar media
- Add distilled water to the solid media and dissolve well
- Dissolve the ingredients by using glass
- Boil the mixture in autoclave

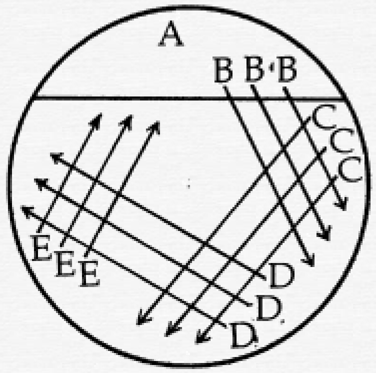
Preparation of culture plates

- Cool the media to about 50 C
- Pour the media into sterilized Petri dish
- Allow the media to cool and solidify keep Petri dishes in an inverted position during storage in refrigerator to prevent condensation of moisture on the plate



Cultivation of bacteria

How to inoculate a plate



- Heat the loop and after cooling it in alcohol transfer a loop-full of culture on the plate (an inoculum)
- Spread bacteria on the solid surface of the plate as in the shape to obtain a single colonies
- The Petri dish is kept in inverted position
- Re sterilize the loop again
- Inoculated media should be incubated at 37 degree as soon as possible

Gram stain

Gram staining (or Gram's method) is an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls.

Staining mechanism

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of cell wall), which stains purple while Gram-negative bacteria have a thinner layer (10% of cell wall), which stains pink.

Gram negative bacteria

Gram-negative bacteria are those bacteria that do not retain crystal violet dye in the Gram staining protocol. In a Gram stain test, a counter stain is added after the crystal violet, coloring all Gram-negative bacteria with a red or pink color.

Examples

- Gram-negative cocci
  - Neisseria gonorrhoeae
- Gram-negative bacilli
  - Helicobacter pylori
  - Vibrio cholera
  - Brucella
  - Salmonella

Gram positive bacteria

Gram-positive bacteria are those that are stained dark blue or violet by Gram staining. This is in contrast to Gram-negative bacteria, which cannot retain the crystal violet stain, instead taking up the counter stain and appearing red or pink.

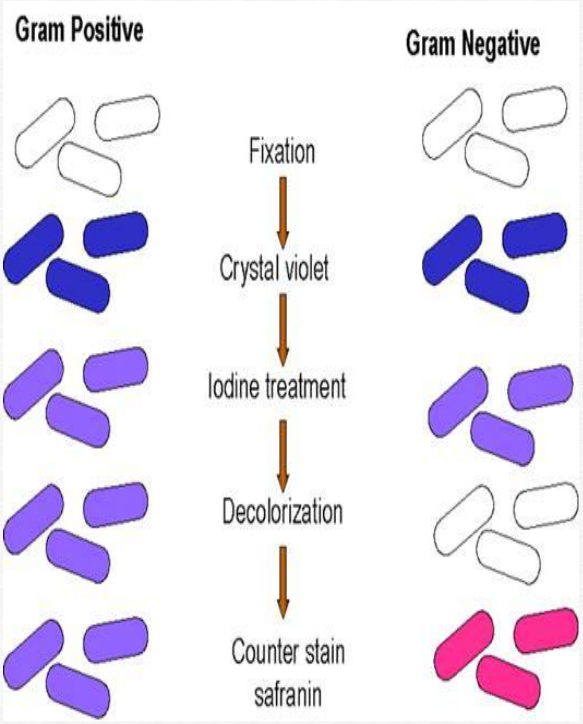
Examples

- Gram-positive cocci
  - Staphylococci
  - Streptococci
- Gram-positive bacilli
  - Bacillus anthracis

- ***Gram stain procedure:***
  - Prepare a clear slide and put one drop of normal saline
  - Take specimen from the colony and mix it well with normal saline.
  - Leave for dryness on air then fix it on burner.
  - Then pour Crystal Violet stain on the slide and leave it for 1 minute.
  - Wash gently with water.
  - Then pour Iodine stain on the slide and leave it for 1 minute.
  - Wash gently with water.
  - Wash with Alcohol to decolorize the slide.
  - Then pour counter stain (fuchsine) on the slide and leave it for 1 minute.

Results under microscope:

Violet cells refer to Gram Positive bacteria (G+ve) and red cells which stained by fuchsine refer to Gram Negative bacteria (G-ve).



Antibiotic sensitivity test

Once the microorganism has been cultured and identified, it is tested for sensitivity to specific antibiotics. Sensitivity testing completes the process known as culture and sensitivity .

purpose

Antibiotic susceptibility testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection in vivo.

Principle

Testing for antibiotic sensitivity is often done by the ***Kirby-Bauer method*** (Agar diffusion method). It is the most commonly used sensitivity test and involves the inoculation of a special agar plate with the organism to be tested. Once the plate has been inoculated, antibiotic disks are placed onto the surface of the agar and the plate is turned upside down and incubated overnight.

The reaction between the antibiotic disks and the organism creates a ***zone of inhibition***, an area of no growth, around each antibiotic disk if the antibiotic is effective against that bacterium. The size of this zone is used to classify the organism as sensitive (S), meaning the antibiotic inhibits growth (the organism is sensitive to the antibiotic); resistant (R), meaning the antibiotic does not inhibit growth (the organism is resistant to the antibiotic); and intermediate (I), meaning the antibiotic inhibits growth somewhat, but not enough to be effective.

Procedures

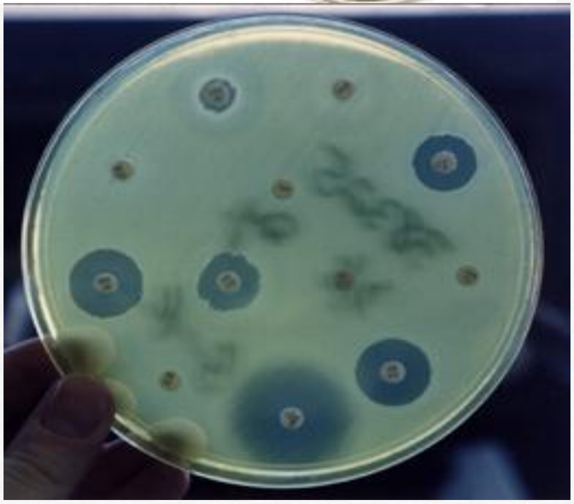
streak plate method

- Plates are dried by opening the plate in the incubator for 10-20 minutes
- Carefully picking one colony by the sterile loop
- Then it is streaked several times along one edge of a Petri dish containing nutrient agar.

  Note: *Swab the surface of the agar completely (you do not want to leave any un-swabbed agar areas at all).*

- After completely swabbing the plate, turn it 90 degrees and repeat the swabbing process.

- Allow the surface to dry for about 5 minutes before placing antibiotic disks on the agar.
- Just quickly pick up the disc and move it to the appropriate place with the sterile forceps.
- Lightly touch each disc with your sterile inoculating loop to make sure that it is in good contact with the agar surface.
- Incubate for 37 degree for 24 hours



Interpretation:

Place the metric ruler across the zone of inhibition, at the widest diameter, and measure from one edge of the zone to the other edge.

Zone diameter is reported as S (sensitive), R (resistant), or I (intermediate).

Antibiotics

The word "antibiotics" comes from the Greek anti ("against") and bios ("life"). Antibiotics are drugs that either destroy bacteria or prevent their reproduction. Antibiotics that kill bacteria are called "bactericidal" and the ones that stop the growth of bacteria are called "bacteriostatic".

Antibiotic classes

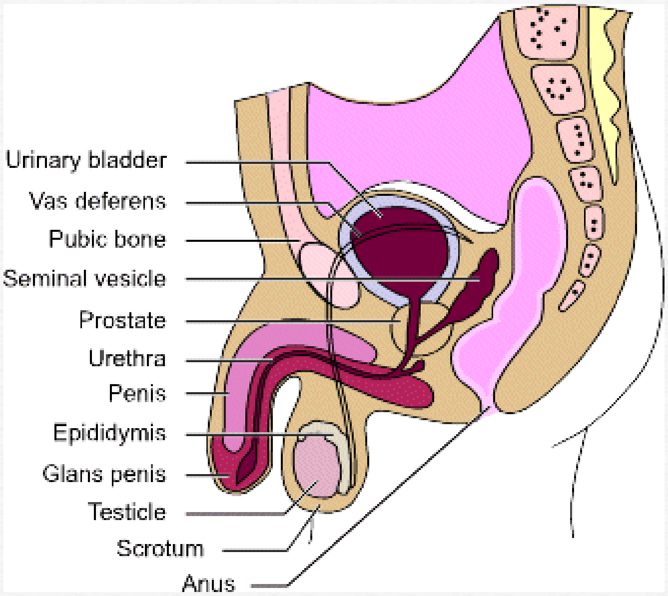
Antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity.

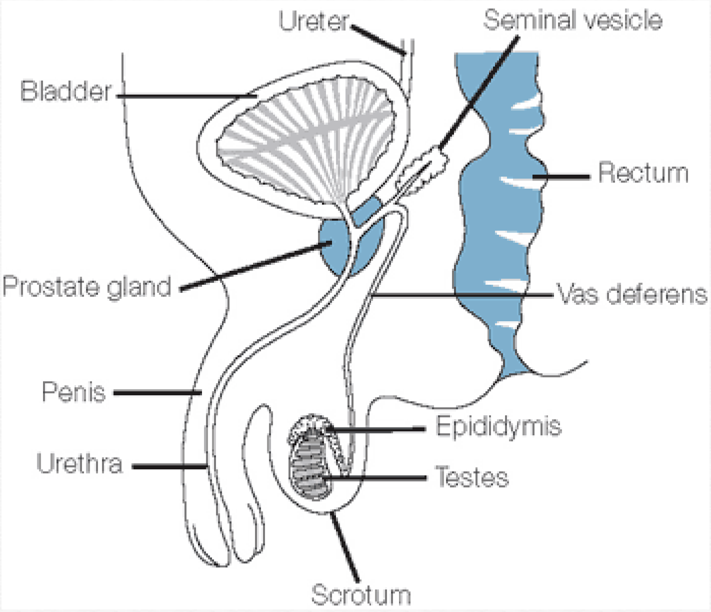
- ***Aminoglycosides***
  Amikin , Gentamicin, Kanamycin, Neomycin
- ***Cephalosporins***
  First generation (Cefadroxil , Cefalexin***)***
  Second generation (Cefuroxime , Cefaclor***)***
  Third generation (Cefoperazone , Cefotaxime (Claforan) , Ceftriaxone (Rocephin)***)***
- ***Macrolides***
  Azithromycin , Erythromycin , Spectinomycin
- ***Nitrofurans***
  Nitrofurantoin
- ***Penicillins & Penicillin combinations***
  Amoxicillin , Ampicillin
  Amoxicillin/clavulanate (Augmentin) , Ampicillin/sulbactam (Unasyn)
- ***Quinolones***
  Ciprofloxacin , Levofloxacin , Ofloxacin
- ***Sulfonamides***
  Trimethoprim-Sulfamethoxazole (Co-trimoxazole)
- ***Tetracyclines***
  Oxytetracycline , Tetracycline
- ***Glycopeptides***
  Vancomycin

Reproduction assays (Andrology)

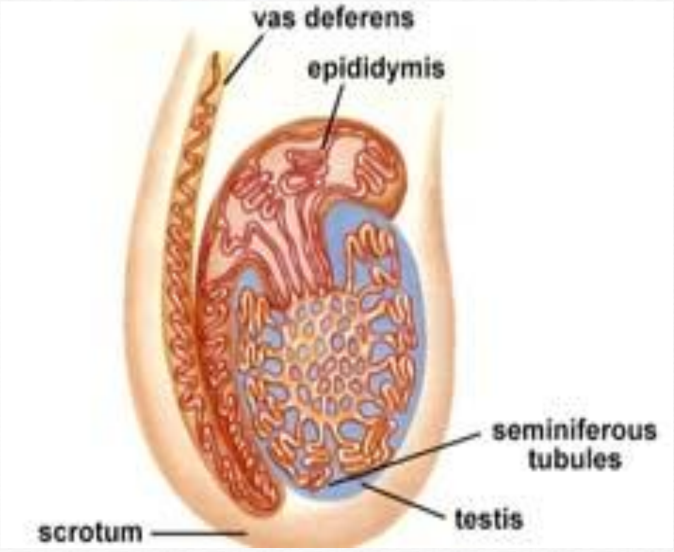
Male reproductive system

The human male reproductive system consists of a number of sex organs that are a part of the human reproductive process. Sex organs are classified as primary sex organ (testes & ducts) and secondary sex organ (external genitalia & accessory glands).





Testicles



- The testes are two small, egg-shaped structures suspended in the scrotum, a loose sac of skin that hangs outside the pelvic cavity between the upper thighs.
- In a male fetus, the testes develop near the kidneys, then descend into the scrotum just before birth (during the last 2 months of fetal development).
- This is required for the production of sperm because internal body temperatures are too high to produce viable sperm.
- Each testis is about 5 cm long and 3 cm in diameter.
- A tough, white fibrous connective tissue capsule, the tunica albuginea, surrounds each testis and extends inward to form septa that partition the organ into lobules.
- There are about 250 lobules in each testis. Each lobule contains 1 to 4 highly coiled seminiferous tubules that converge to form a single straight tubule, which leads into the short efferent ducts exit the testes.

Seminiferous tubule

Seminiferous tubules are located in the testicles, and are the specific location of meiosis, and the subsequent creation of gametes, namely spermatozoa.

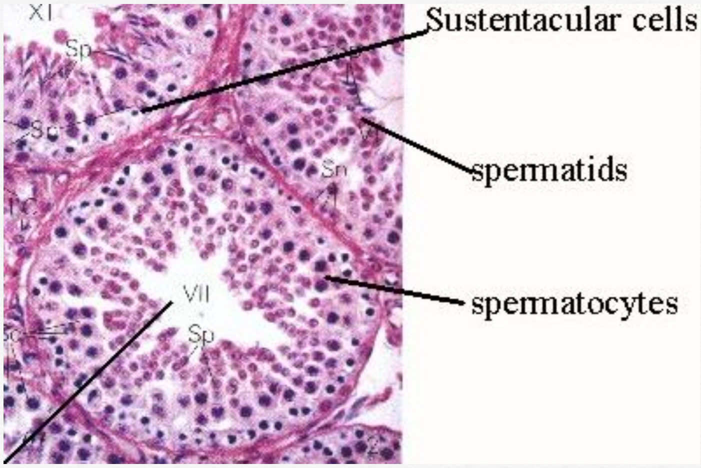
The Seminiferous tubules consist of:

- **sustentacular or Sertoli cells:**

  Which are tall, columnar type cells that line the tubule and support and nourish the other cells.

  Functions

  - Secretes ABP which is essential for testosterone action
  - Translocate the differentiated spermatogenic cells



- **spermatogenic cells:**

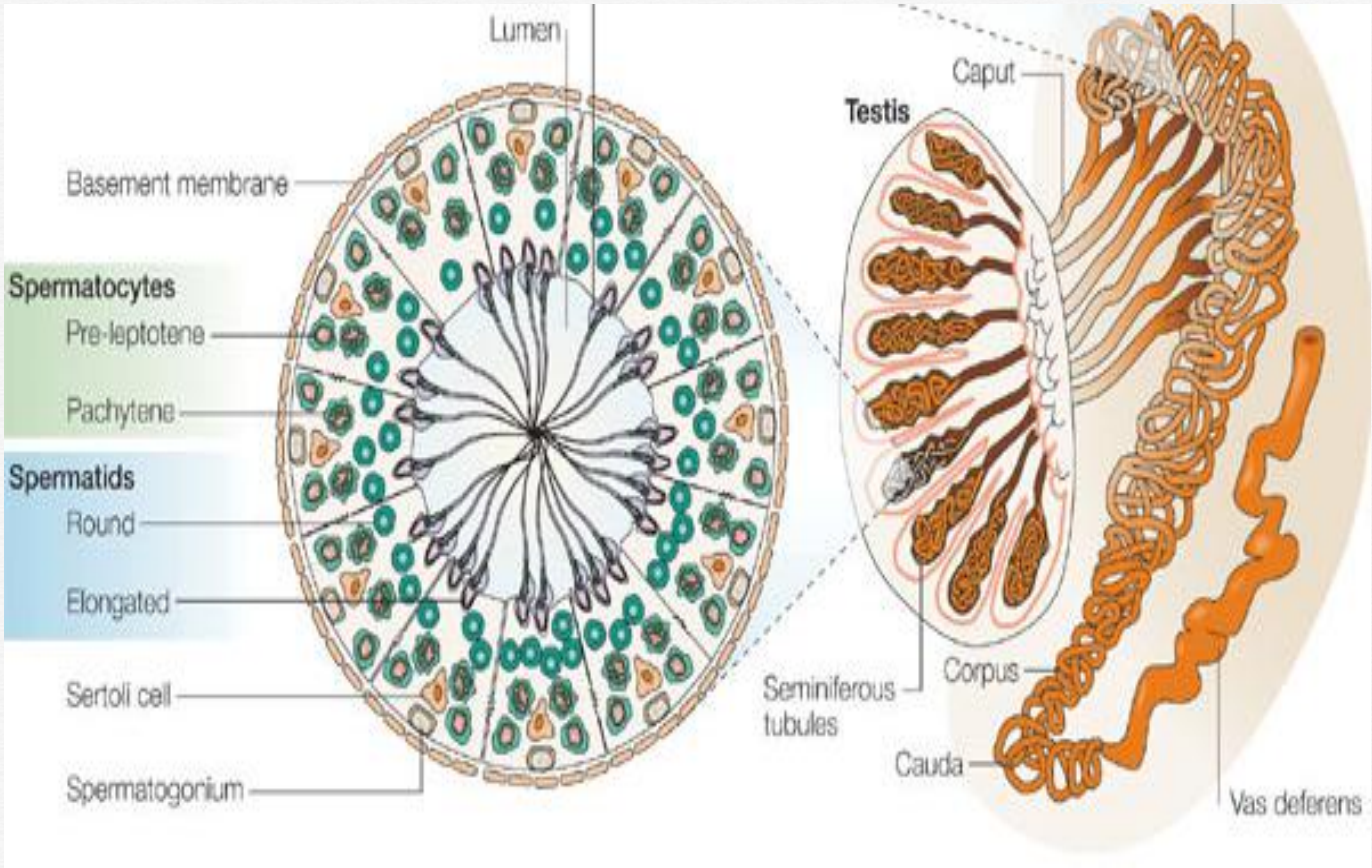
  In between the Sertoli cells are spermatogenic cells, which differentiate through meiosis to sperm cells.

  Functions

  - The cells that differentiate into sperms

- **Interstitial cells (cells of Leydig):**

  Which produce male sex hormones, are located between the seminiferous tubules within a lobule.



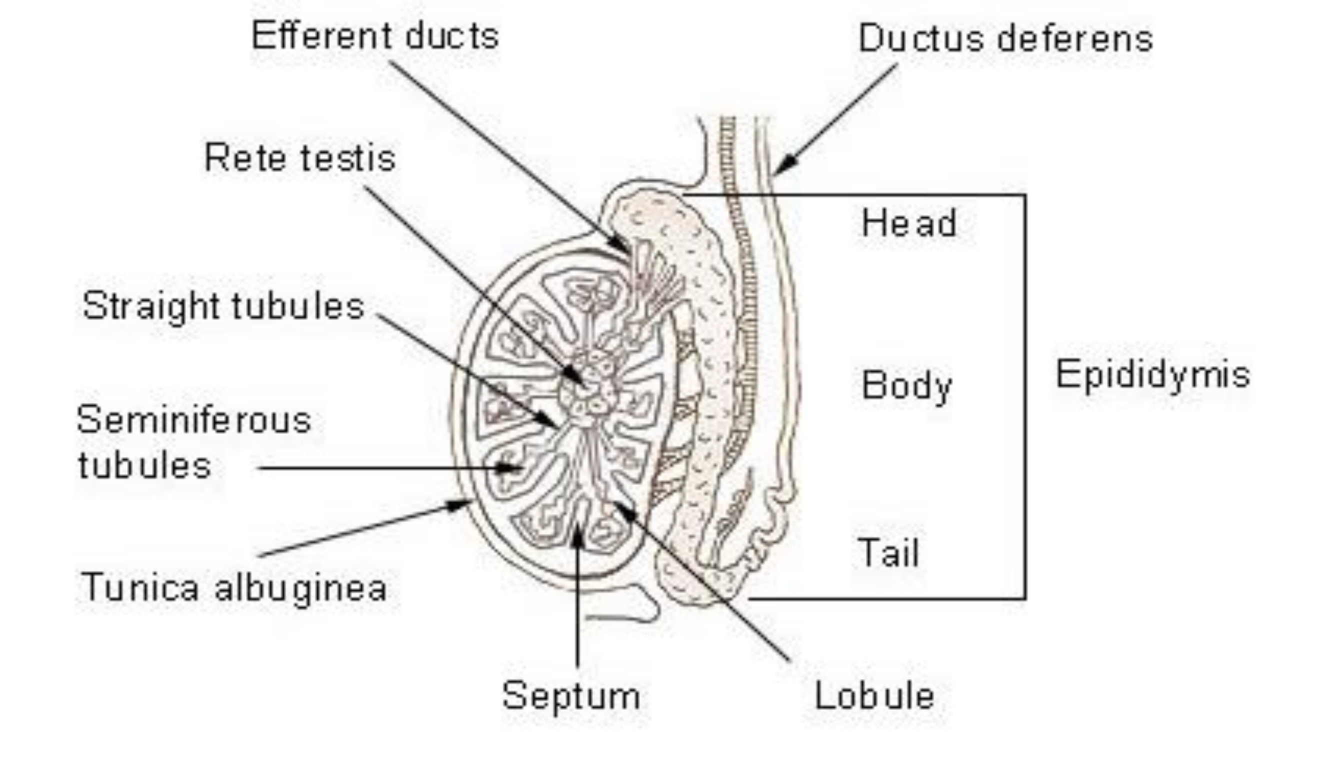
Duct system

Epididymis

- Sperm leave the testes through a series of efferent ducts that enter the epididymis.
- Each epididymis is a long (about 6 meters) tube that is tightly coiled to form a comma-shaped organ located along the superior and posterior margins of the testes.
- When the sperm leave the testes, they are immature and incapable of fertilizing ova. They complete their maturation process and become fertile as they move through the epididymis (this takes about two weeks).
- Mature sperm are stored in the lower portion, or tail, of the epididymis.

Capaitation

A series of biochemical changes occurs in sperm to attain their full capacity for fertilization



Vas deferens

- The ductus deferens, also called the vas deferens, extends from each epididymis upward over the top of the bladder, then down its back side.
- The paired ducts measure between 40 and 45 centimeters in length.
- Their smooth muscular walls move the sperm along through peristaltic contractions

Ejaculatory duct

- The ejaculatory duct is a short passageway that is formed by the union of a ductus deferens and the duct of a seminal vesicle.
- Each ejaculatory duct measures just under 2.5 centimeters in length
- Both ejaculatory ducts empty sperm (from the ductus deferens) and fluid (from the seminal vesicle) into the single urethra

Urethra

- The urethra extends from the base of the urinary bladder to the tip of the penis, a distance of 15 to 20 centimeters.
- In males, the urethra serves both the reproductive and urinary systems. It transports sperm (with its fluid) and urine to the body exterior, but never both at the same time.
- When sperm enters the urethra from the ejaculatory ducts, a sphincter or ring of muscle at the junction of the bladder and urethra closes, keeping urine in the bladder (and also preventing sperm from entering the bladder)

External genitalia

The scrotum and penis are the male genitalia that hang outside the body.

Scrotum

- The scrotum is a loose sac of skin that hangs outside the pelvic cavity between the upper thighs. It is divided into two compartments, each holding a testis.
- The scrotum holds the testes away from the body since normal body temperature is too warm for sperm to be produced (The temperature inside the scrotum is a few degrees cooler than inside the rest of the body).
- If the external temperature becomes very cold, muscles in the scrotum pull the testes closer to the body, maintaining the proper temperature for sperm production.

Penis

The penis is the male copulatory organ.

- When the male becomes sexually aroused, the penis becomes erect and ready for sexual intercourse.
- The two main parts of the penis are the glans (enlarged tip) and shaft (body).
- A fold of skin called the prepuce or foreskin covers the glans.

  circumcision

  It is common practice in certain cultures and religions to remove the foreskin surgically soon after birth, a procedure called a circumcision.
- The shaft contains columns of erectile tissue(corpus cavernusa and corpus spongiosum). Normally, this spongy tissue is not filled with much blood. During sexual arousal, however, blood flow to the tissue increases. The penis, engorged with blood, becomes longer, wider, and rigid. This event, called an erection, allows the penis to enter the female vagina and deliver semen to the female's reproductive tract.

Mechanism of erection

- Erection is initiated by Parasympathetic branches extend into the arteries supplying the erectile tissue
- Upon stimulation, these nerve branches release acetylcholine, which, in turn causes release of nitric oxide from endothelial cells in the penis arteries.
- Nitric oxide diffuses locally into adjacent smooth muscle cells, and binds with intracellular guanylate cyclase, which serves as a physiologic "receptor" for the molecule.
- This binding induces a conformational change of guanylate cyclase, activating the enzyme so that it catalyzes the conversion of guanosine triphosphate (GTP) to cGMP. cGMP then operates through a cGMP-dependent protein kinase to regulate the contractile state of the smooth muscle of the arteries, acting as a vasodilating agent
- The arteries dilate, filling the corpora spongiosum and cavernosa with blood.

- Once blood start flow into the penis nitric oxide is continuously activated so more nitric oxide is released and more tissue relaxed and more blood come in so sustained erection is achieved

Viagra

Sildenafil citrate is a drug used to treat erectile dysfunction and pulmonary arterial hypertension (PAH). It was developed and is being marketed by the pharmaceutical company Pfizer.

Mechanism of viagra action

- Inhibition of cGMP dependent PDE so potintiate the effect of NO mediated action on target cells leading to smooth muscle relaxation (vasodilation) of arteries. This smooth muscle relaxation leads to vasodilation and increased inflow of blood into the spongy tissue of the penis, causing an erection.
- Sildenafil Citrate binds with the active site of the PDE enzyme, thus preventing it from hydrolysing cGMP. This allows the produced cGMP to remain in its active form for longer and hence have a larger vasodilatory effect.

Accessory glands

Three accessory glands produce secretions that combine with sperm to create a whitish, somewhat sticky mixture called semen.

Those secretions (the fluid part of semen) are known collectively as seminal fluid

The seminal vesicles

- The first accessory glands to add secretions to sperm
- Located at the base of the bladder, their ducts join with the paired ductus deferens to form the ejaculatory ducts.
- Their thick, yellowish secretion, which makes up about 60 percent of the seminal fluid, contains fructose, which provides an energy source for the sperm; prostaglandins, which contribute to the mobility and viability of the sperm; and proteins that cause slight coagulation reactions in the semen after ejaculation.

The prostate gland

- In the adult, the prostate gland is a single, rounded gland that has a diameter of about 4 centimeters and lies immediately below the urinary bladder, surrounding the upper part of the urethra
- The prostate gland is comprised of 30–50 glands arranged in acini, which empty into the prostatic urethra, the tube that connects the prostate gland with the bladder urethra.
- The prostate gland is divided into three zones: the peripheral zone, transition zone and central zone. The whole organ is encapsulated in a fibrous prostatic capsule.

Function

- The main function of the prostate gland is to secrete a milky, alkaline fluid (one of the components of semen makes up about 30 percent of the seminal fluid) into the urethra at the point of ejaculation. The prostate fluid helps to nourish and protect the sperm during intercourse.

The bulbourethral glands

The paired bulbourethral glands (***Cowper's gland***) are small, about the size of a pea, and located near the base of the penis.

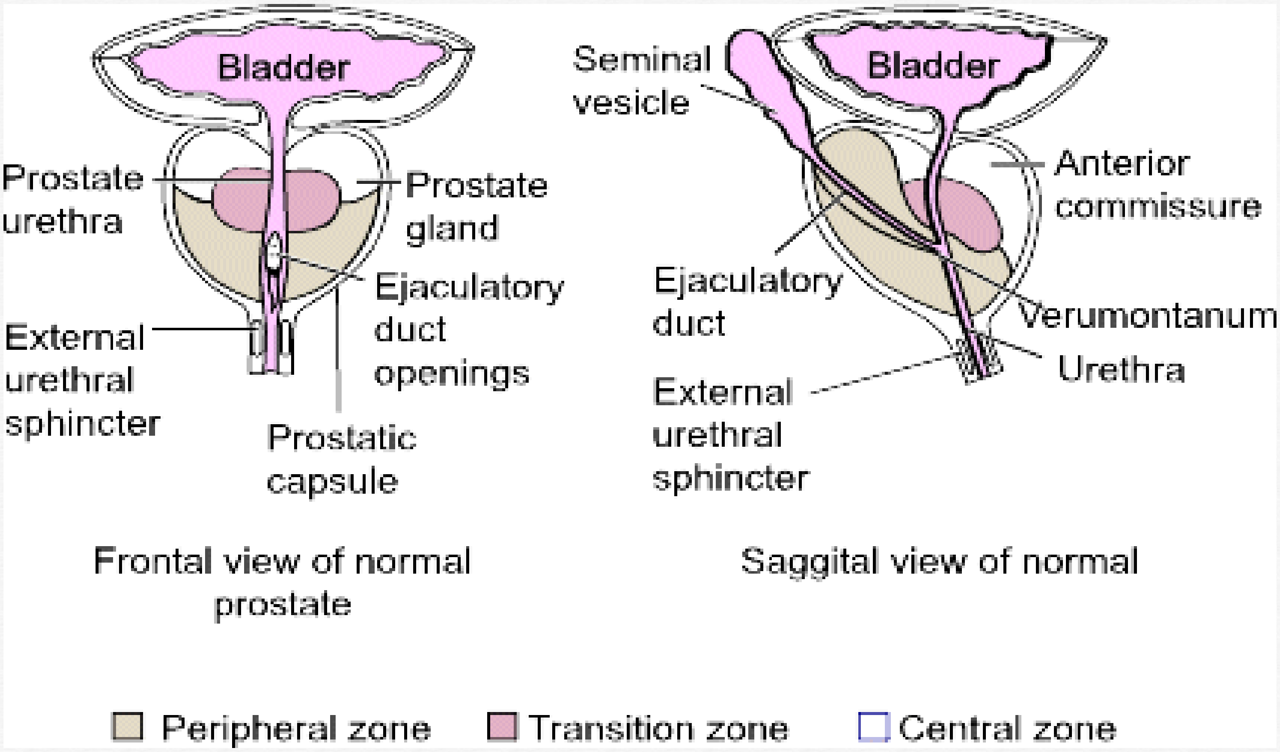
A short duct from each gland enters the proximal end of the penile urethra.

Their secretion—a thick, clear mucus—enters the urethra when a man becomes sexually aroused before the secretions of the other accessory glands.

Function

- The secretion coats the lining of the urethra and cleanses it of any traces of acidic urine that might be present.
- It also serves as a lubricant during sexual intercourse.

Note: *The secretion of all three accessory glands is slightly alkaline. This is important because the bacteria present in the female vagina create an acidic environment there. The alkaline seminal fluid neutralizes the acid in the vagina, allowing sperm in the semen to exist and move in the vagina so fertilization may take place.*



Spermatogenesis

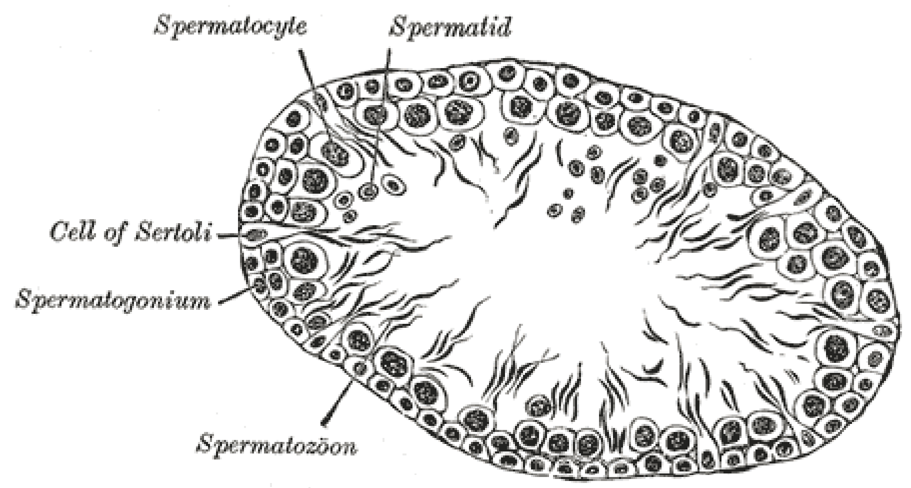
Spermatogenesis is the process by which male spermatogonia develop into mature spermatozoa.

Spermatocytogenesis

- Early in embryonic development, primordial germ cells enter the testes and differentiate into spermatogonia, immature cells that remain dormant until puberty.
- Spermatogonia are diploid cells, each with 46 chromosomes (23 pairs) located around the periphery of the seminiferous tubules.
- At puberty, hormones stimulate these cells to begin dividing by mitosis.
- Some of the daughter cells produced by mitosis remain at the periphery as spermatogonia.
- Others are pushed toward the lumen, undergo some changes, and become primary spermatocytes.
- Because they are produced by mitosis, primary spermatocytes, like spermatogonia, are diploid and have 46 chromosomes.
- Each primary spermatocytes goes through the first meiotic division, meiosis I, to produce two secondary spermatocytes, each with 23 chromosomes (haploid).

- In the second meiotic division, meiosis II, each secondary spermatocyte divides to produce two spermatids (with 23 chromosomes).

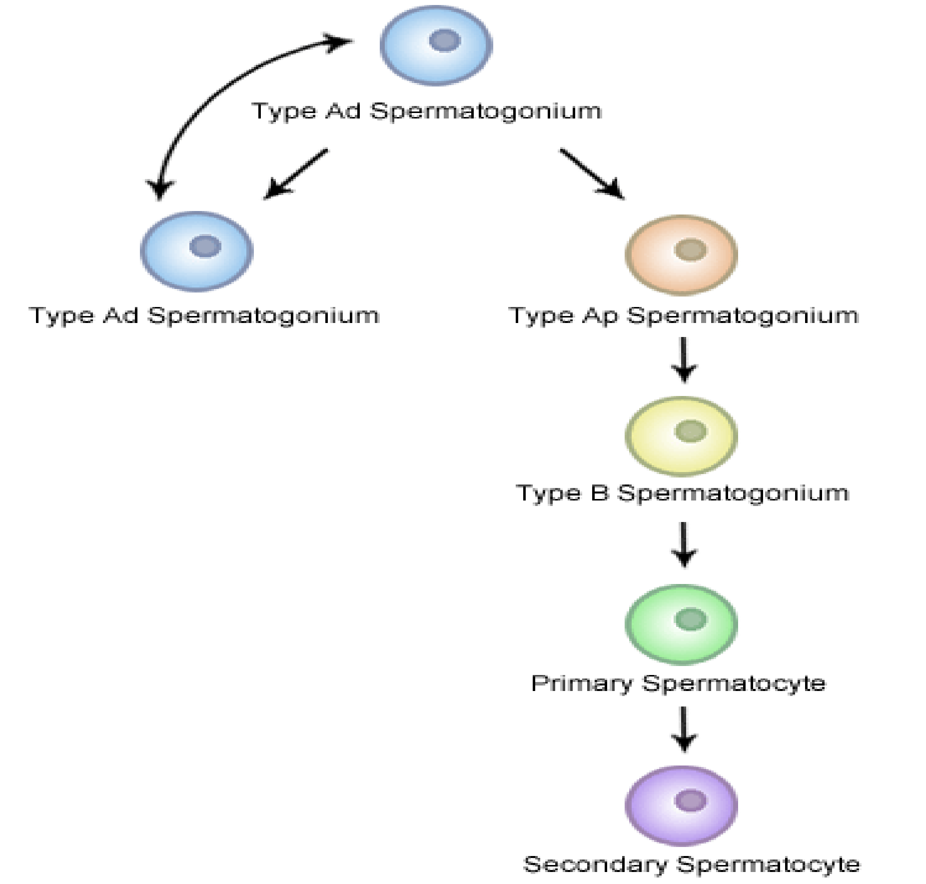
Note: *As a result of the two meiotic divisions, each primary spermatocyte produces four spermatids.*



Spermiogenesis

It is the metamorphosis of spermatid to sperm
The final step in the development of sperm is called spermiogenesis.
In this process, the spermatids formed from spermatocytogenesis become mature spermatozoa, or sperm.
The sperm are released into the lumen of the seminiferous tubule and leave the testes.
They then enter the epididymis where they undergo their final maturation and become capable of fertilizing a female gamete.

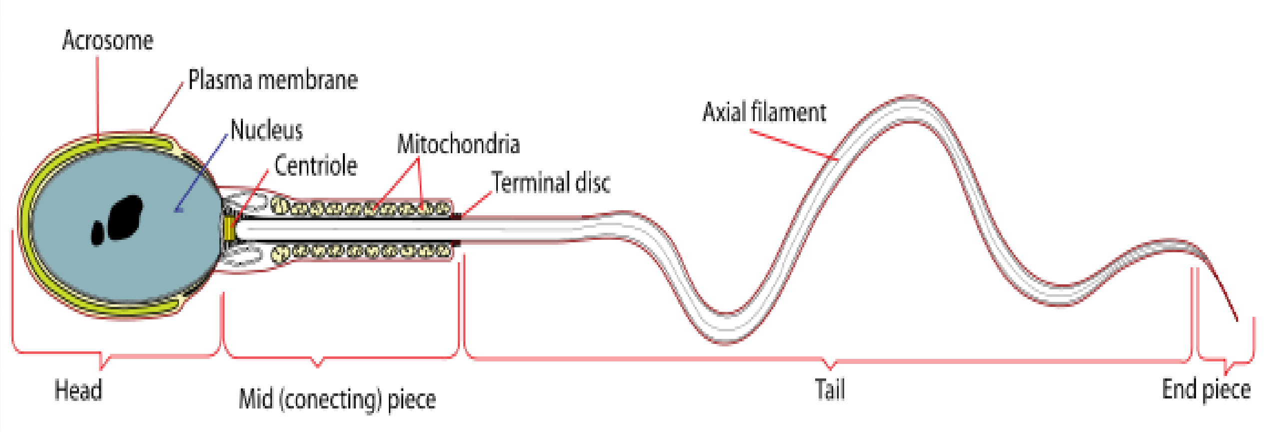
Note: *Sperm production begins at puberty and continues throughout the life of a male. The entire process, beginning with a primary spermatocyte, takes about 74 days. After ejaculation, the sperm can live for about 48 hours in the female reproductive tract.*



Sperm structure

The mature sperm cell has a head, midpiece, and tail.

- ***The head***, also called the nuclear region, contains the 23 chromosomes surrounded by a nuclear membrane. The tip of the head is covered by an acrosome, which contains enzymes that help the sperm penetrate the female gamete.
- ***The midpiece***, metabolic region, contains mitochondria that provide adenosine triphosphate (ATP).
- ***The tail***, locomotor region, uses a typical flagellum for locomotion. Sperm tail is move by sliding model of dynine arm



Hormonal control

At puberty (usually occurring between the ages of twelve and sixteen in boys), the anterior portion of the pituitary gland releases luteinizing hormone.

This hormone activates the Leydig cells to begin secreting testosterone.

The anterior pituitary then releases follicle-stimulating hormone, which stimulates the cells of the seminiferous tubules to begin producing sperm and FSH stimulate sertoli to secrete (androgen binding protein)ABP.

.

Negative feedback control

- Testosterone controls its own release through a negative feedback effect on the hypothalamic-pituitary-testicular axis.
- When testosterone levels in the bloodstream are raised, the hypothalamus reduces the secretion of LHRH, which inhibits the secretion of LH from the pituitary gland. The overall effect is to reduce the amount of LH acting on the Leydig cells, thus reducing testosterone secretion.

Routine semen analysis

Semen is a viscous, whitish liquid that contains sperm and the products from several glands. It is normally fairly thick after ejaculation and then thins, liquefying within 10 to 30 minutes. Sperm are reproductive cells in semen that have a head and a tail. Each sperm contains one copy of each chromosome (all of the male's genes). Sperm are motile, normally moving forward through the semen. Inside a woman's body, this property enables them to travel to and fuse with the female's egg, resulting in fertilization. In each semen sample, there are millions of sperm and varying amounts of fructose, buffers, coagulating substances, lubricants, and enzymes that are intended to support the sperm and the fertilization process.

Why it is done?

A semen analysis is performed when a physician thinks that a person or couple might have a fertility problem. At least 10%-20% of married couples experience problems conceiving. Male factors are implicated about 30% of the time and combined male and female factors about 20% of the time.

Collection precautions

- Avoid sexual activity for 3-5 days before analysis

  Note: *Do not avoid sexual activity for more than 2 weeks because this can result in less active sperms*

- Semen is provided by ejaculating into a clean sample cup
- The whole sample must be collected (majority of sperms is in the first portion of ejaculate)
- Do not use water or detergents for collection

  Note: *Coitus interrupts is not recommended for collection (due to low sperm count and reduced motility by acidic pH)*

- if you collect the sample at home it must be delivered to lab with in 20 minutes (not allow to get cold or hot (exposure to cold will cause incorrectly low motility)

  Note: *If urine and semen sample is ordered , urine must be collected first*

Routine semen examination

Put the sample in 37ºC incubator at once then after 20 min Incubation perform the test as Fellow:

Physical examination

Volume

Procedure

Using Volumetric pipette

Normal

2 - 6 ml

Abnormalities

Low Semen Volume

This can be seen in cases of problems with accessory gland , obstruction , or retrograde ejaculation

Note: *Total semen volume also influences fertility. If the volume is too small, say under one milliliter, you may not have enough fluid to bring the sperm in contact with wife's*

cervix (the entrance of her womb). In addition, an insufficient quality of protective semen will expose sperms to the acid, sperm-killing environment of vagina.

High Semen Volume

found with varicocele or long period of sexual abstance

► Color

Procedure

By Naked eye observation

Normal

Grayish white

Abnormalities

- Yellow color may indicate pyospermia , jaundice, or prolonged sexual abstance
- Brownish color may indicate hematospermia

► Reaction

Procedure

Using red Litmus paper

Normal

Alkaline to protects semen from vaginal acidity

Abnormalities

Increased semen pH

Due to infection with decreased prostatic secretion

Decreased semen pH

- Acidic pH suggests problems in seminal vesicles function
- Also due to lactic acid built up with high sperm count

Note: *Acidic pH with azospermia suggests obstruction of ejaculatory duct*

► Consistency

Procedure

By glass rod , the mucus threads should not exceeds 2 cm

Viscosity graded ax watery, viscous, highly viscous, gel like

Normal

viscous

Abnormalities

- Watery viscosity usually points to low cell content
- Increased viscosity may due to prostatic dysfunction from chronic inflammation

Effects of high viscosity

Semen viscosity also affects fertility potential. If coagulated semen does not liquefy within an hour of ejaculation, sperm may be trapped in the cottage cheese-like jelly and this cause poor invasion of cervical mucus.

► Liquefaction time

Procedure

Semen is coagulated after contact with air by the action of prostatic clotting enzymes and fibrinogen from seminal vesicles then after 20 minutes it liquefy by prostatic fibrinolysis enzymes from prostate

Observe liquefaction after 20 minutes and if not Liquefied complete incubation till or for 3 hours

Normal

Less than 20 minutes

Abnormalities

- Cause of high liquefaction time : high semen viscosity
- Causes of low liquefaction time: May suggests functional disturbance of the prostate

Generally no coagulation through 30 minutes may indicate infertility

Microscopic examination

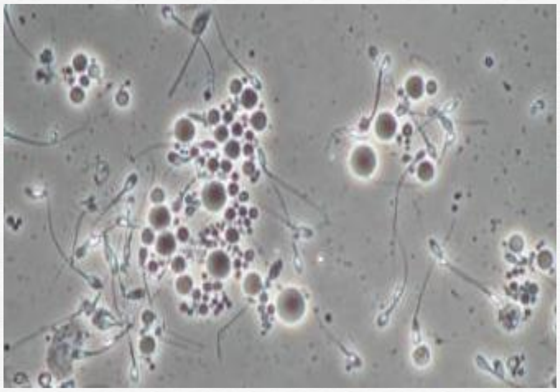
It is done after semen liquefaction and slide is examined under 10 an 40X , it must be done on warm slide because motility and velocity depends on temperature

Cells in semen

Pus cells

Procedure

Observation and counting under high power field



Normal

Less than 3/HPF

Abnormalities

Pyospermia (increased Pus cells in semen) usually indicate infections or inflammation of testes or prostate gland.

Effects of Pyospermia

WBCs stimulate the release of (reactive oxygen species) ROS so inhibiting sperm motility and affect fertility.

Red blood cells

Procedure

Observation and counting under high power field

Normal

Less than 3/HPF

Abnormalities

Hemospermia (increased RBCs in semen) may indicate Inflammation of tests (orchitis), Prostatits, testicular congestion or prostatic cancer.

Signs associated with Hemospermia

- Pain with ejaculation
- Tenderness in scortum
- Swelling in scortum
- Lower back pain

Spermatogonic cells

spermatogenic cells, differentiate through sperm cells.

Procedure

Observation and counting under high power field, it must be differentiated from pus cells (its volume is larger than pus)

Sperm parameters

Sperm count:

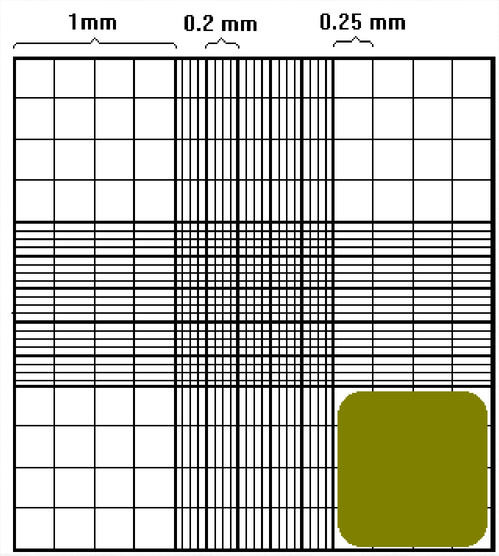
Procedure

Prepare the following semen dilution 1/20:

380 µl distilled water + 20 µl semen sample

Counting:

- *Using Heamocytometer (on WBCs count squares)*
  Sperm count = Count in 4 squares X 50,000
- *Using Heamocytometer (on RBCs count squares)*
  Sperm count = Count in 5 squares X 1000,000



Sources of errors:

- Sample should be mixed in the original container and after dilution.
- Distribution of sample on slide must be correct.

Note: *In cases of azospermia centrifuge semen* then observe sperm count under HPF.

Normal

Sperm concentration (also called sperm density) is measured in millions of sperm per milliliter (ml) of semen. Normal is 60-120 million (20 million is enough for fertilization)

Abnormalities

- ***Causes of low sperm count (Oligospermia)***
  - Tests over heating as with high fever or hot bath
  - Smoking may affect sperm count
  - Exposure to environmental toxins as DDT
  - Radiation treatment.
  - Infections. Infection of uro-genital tract may affect sperm production. Repeated bouts of infections are one of the common causes associated with male infertility.
  - ***Varicocele affect sperm production:*** varicocele is dilatation of scrotal vein in the scrotum that leads to rise in temperature of testis and raise testicular temperature, resulting in less sperm production & death of whatever sperms are produced.
  - ***Undescended testicle (cryptorchidism):*** Undescended testis is a condition when one or both testicles fail to descend from the abdomen into the lower part of scrotum during fetal development. Undescended testicles can lead to less sperm production. Because the testicles temperature increase due to the higher internal body temperature compared to the temperature in the scrotum, sperm production may be affected.
- ***Causes of zero sperm count (azospermia)***
  - Congenital absence of vasdeference (CAVD)
  - Y-chromosome microdletion this mutation affect sperm production
  - ***Klinefelter's syndrome***: in this disorder of the chromosomes, a man has two X chromosomes and one Y chromosome instead of one X and one Y. This causes abnormal development of the testicles, resulting in low or absent sperm production. Testosterone production also may be lower and FSH level is high due to germinal epithelial damage.

► Abnormal forms

Procedure

Morphology analysis is the study of the size, shape, and appearance of the sperm cells. The analysis evaluates the structure of 200 sperm, and any defects are noted.

A normal-looking sperm has an oval head and a tail seven to fifteen times longer than the head.

Abnormal forms may include defective heads, middles, tails, and immature forms.

Normal

The World Health Organization says good quality semen should contain more than 30 percent normal sperm morphology.

Abnormalities

Teratozoospermia means that sperm carry morphological defects which may indicate impaired spermatogenesis or may caused in cases of varicocele.

Generally the more abnormal sperm that are present, the lower the likelihood of fertility.

► Sperm motility

Procedure

Motility is the percentage of moving sperm in a sample and an evaluation of their rate and direction of travel.

Sperm motility is seen directly after liquefaction

- Total motility after 20 hour.
- Total motility after 1 hour.
- Total motility after 2 hours.
- Total motility after 3 hours from ejaculation.

Normal

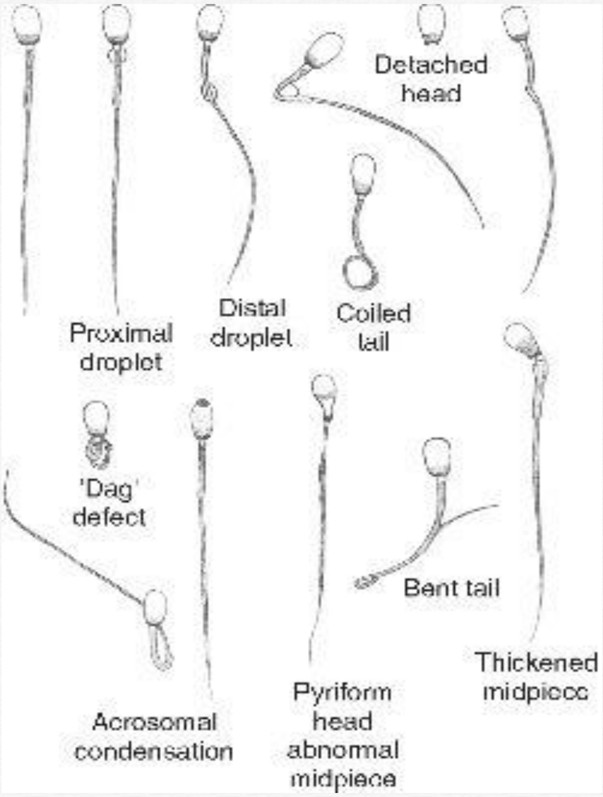
At least 50% or more should be motile one hour after ejaculation, and they should be moving forward in a straight line with good speed.

Total motile count:

This represent the total number of sperms that is capable of movement.

Total motile count = volume X sperm count X % motility

Normally it must be 8 million/ml or higher



Abnormalities

Asthenospermia means a low Sperm Motility which may be caused by:

- Defects in spermatogensis
- Varicocele
- Epididymal dysfunction (differentiation into motile sperm occurs in epiddems)
- Prostatits (inflamed prostate gland)

► Type of motility

Procedure

Observation under microscope and classification of sperm motility as follow:

- ***Rapid progressive (active):*** Rapid straight forward movement.
- ***Sluggish (weak):*** Slow irregular movement (Backward – In circles – forward)
- ***Non progressive:*** Movement in situ
- ***Immotile:*** No Movement (may be dead sperms)

Note: *prolonged exposure of microscopic light will diminish motility*

Abnormalities

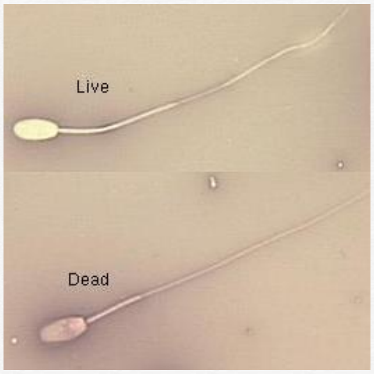
low sperm motility is associated with infertility because sperm can not penetrate through cervical mucus

Note: *In some cases the sperm may be alive sperm but lack one or more factor that is necessary for motility so in this case sperm viability test should be done*

Sperm viability

Procedure

- Using Eosin stain: Put one drop of semen + one drop of Eosin stain.
- Examine under microscope
  - If sperm takes the dye it will appear with red head and this indicate a dead sperm.
  - If sperm doesn't takes the dye it will appear with normal head and this indicate alive sperm.



Abnormalities

Necrozoospermia means dead Sperms

When semen more of dead sperms this condition is abnormal. This is one of the common causes of male factor infertility. This is also one of the most common semen abnormalities in men.

The various causes of dead sperms are as follows:

- Varicocele may leads to death of sperms that is produced.
- Autoimmunity i.e. presence of Antisperm antibody. These Antisperm antibodies bind with sperms & either make them less motile, totally immotile or even dead.
- Partial Spermatogenic arrest due to interruption of the complex process of germ cell differentiation from spermatid level to the formation of mature spermatozoa results in decreased sperm count. Its diagnosis is made by testicular biopsy. This is found in up to 30% of all cases of dead sperm patients.

Reproduction assays (Gynacology)

Menstrual cycle

The menstrual cycle is a cycle of physiological changes that occurs in fertile females. The menstrual cycle is divided into several phases. The average length of each phase is shown below:

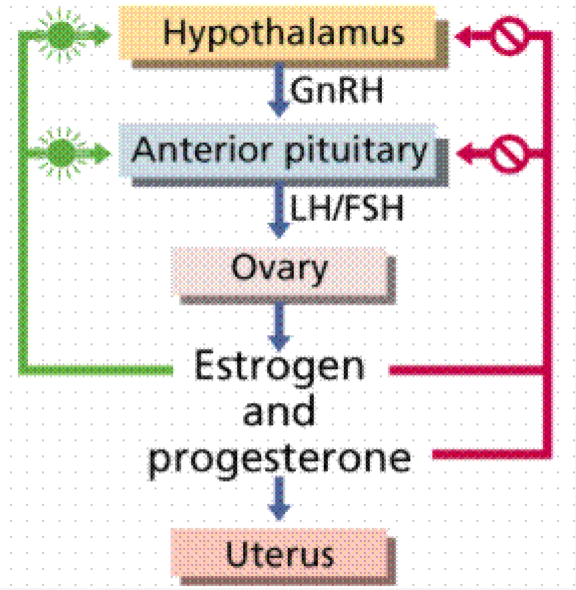
Name of phase	Average start day assuming a 28-day cycle	Average end day
menstrual phase	1	4
follicular phase (also known as proliferative phase)	5	13
ovulation (not a phase, but an event dividing phases)	12	16
luteal phase (also known as secretory phase)	15	28

Follicular phase

- Through the influence of a rise in follicle stimulating hormone (FSH) during the first days of the cycle, a few ovarian follicles are stimulated.
- All but one of these follicles will stop growing, while one dominant follicle in the ovary will continue to maturity.
- The follicle that reaches maturity is called a tertiary, or Graafian, follicle, and it forms the ovum.
- As they mature, the follicles secrete increasing amounts of estrogen.
- The estrogens initiate the formation of a new layer of endometrium in the uterus

Ovulation

- During the follicular phase, estrogen suppressed production of luteinizing hormone (LH) from the anterior pituitary gland.
- When the egg has nearly matured, levels of estrogen reach a threshold above which they stimulate production of LH.
- In the average cycle this LH surge starts around cycle day 12 and may last 48 hours.
- The release of LH matures the egg and weakens the wall of the follicle in the ovary, causing the fully developed follicle to release its ovum.
- After being released from the ovary, the egg is swept into the fallopian tube by the fimbria



Luteal phase

- An important role is played by the corpus luteum, the solid body formed in an ovary after the egg has been released from the ovary into the fallopian tube.
- The pituitary hormones LH cause the corpus luteum, to produces significant amounts of hormone, progesterone.
- Progesterone plays a vital role in making the endometrium receptive to implantation and

supportive of the early pregnancy; it also has the side effect of raising the woman's basal body temperature.

- Progesterone also suppresses production of the LH that the corpus luteum needs to maintain itself. Consequently, the level of LH fall quickly over time, and the corpus luteum subsequently atrophies.
- Falling levels of progesterone trigger menstruation and the beginning of the next cycle.

Note: *The loss of the corpus luteum can be prevented by fertilization of the egg; the resulting embryo produces human chorionic gonadotropin (hCG), which is very similar to LH and which can preserve the corpus luteum.*

Menstruation

- Menstruation is also called menstrual bleeding, menses, catamenia or a period. The flow of menses normally serves as a sign that a woman has not become pregnant. (However, this cannot be taken as certainty, as a number of factors can cause bleeding during pregnancy)
- Normal, regular menstruation lasts for a few days (usually 3 to 5 days, but anywhere from 2 to 7 days is considered normal).
- The average blood loss during menstruation is 35 milliliters with 10–80 ml considered normal.

Note: *Because of this blood loss, women are more susceptible to iron deficiency than are men*

Fertile window

The most fertile period (the time with the highest likelihood of pregnancy resulting from sexual intercourse) covers the time from some 5 days before until 1–2 days after ovulation. In an average 28 day cycle with a 14-day luteal phase, this corresponds to the second and the beginning of the third week.

Amenorrhoea

Amenorrhoea, is the absence of a menstrual period in a woman of reproductive age.

Classification of amenorrhoea

There are two types of amenorrhea: primary and secondary amenorrhea. Primary amenorrhoea is the absence of menstruation in a woman by the age of 16.

Primary amenorrhoea

Primary amenorrhoea is the absence of menstruation in a woman by the age of 16
Due to congenital or hereditary defects for example receptor abnormalities for hormones FSH and LH.

Secondary amenorrhoea

Is where an established menstruation has ceased which may caused by:

- Hypothalamic-pituitary dysfunction
- Exercise amenorrhoea, related to physical exercise (amenorrhea has been shown to be directly attributable to a low energy availability. Many women who exercise at a high level do not take in enough calories to expend on their exercise as well as to maintain their normal menstrual cycles)
- Stress amenorrhoea,
- Hyperprolactinemia
- Thyroid dysfunction

Fertilization

Fertilization is the union of a human egg and sperm, usually occurring in the fallopian tube.
The process involves a sperm fusing with an ovum, which eventually leads to the development of an embryo.
It is when first of all the acrosome at the head tip produces enzymes, which cuts through the outer jelly coat of the egg. After that has happened, the sperm plasma fuses with the egg's plasma membrane. Finally, the Head disconnects with the body, and the egg can now travel down the Fallopian tube to reach the womb, where the baby grows.

Implantation

Within 9 days after fertilization, the egg moves down the fallopian tube into the uterus and attaches (implants) to the wall of the uterus. When the fertilized egg implants, the placenta starts to develop and begins to release hCG into the woman's blood. Some of this hCG also gets passed in her urine.

The placenta

The placenta is an organ unique to mammals that disconnects the developing fetus to the uterine wall. The placenta supplies the fetus with oxygen and food, and allows fetal waste to be disposed of via the maternal kidneys.

Human chorionic gonadotropin

is a glycoprotein hormone produced in pregnancy that is made by the developing embryo soon after conception and later by the placenta.

Structure

Human chorionic gonadotropin is a glycoprotein composed of 244 amino acids.
It is heterodimeric, with an α (alpha) subunit identical to that of luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and β (beta) subunit that is unique to hCG.

Function

Its role is to prevent the disintegration of the corpus luteum of the ovary and thereby maintain progesterone production that is critical for a pregnancy in humans.

Reference levels

The following is a list of serum hCG levels. (LMP is the last menstrual period.)

3 weeks since LMP: 5 - 50 mIU/ml
4 weeks since LMP: 5 - 426 mIU/ml
5 weeks since LMP: 18 - 7,340 mIU/ml
6 weeks since LMP: 1,080 - 56,500 mIU/ml
7 – 8 weeks since LMP: 7, 650 - 229,000 mIU/ml
9 – 12 weeks since LMP: 25,700 - 288,000 mIU/ml
13 – 16 weeks since LMP: 13,300 - 254,000 mIU/ml
17 – 24 weeks since LMP: 4,060 - 165,400 mIU/ml
25 – 40 weeks since LMP: 3,640 - 117,000 mIU/ml
Non-pregnant females: <5.0 mIU/ml
Postmenopausal females: <9.5 mIU/ml

Note: *In the first few weeks of pregnancy, the amount of hCG in the urine rises rapidly, doubling every 2 to 3 days.*

Note: *Failure to increase normally may indicate an increased risk of miscarriage or a possible ectopic pregnancy.*

Pregnancy staging

Pregnancy is typically broken into three periods, or trimesters, each of about three months.

First trimester

- Pregnancy is often defined as beginning when the developing embryo becomes implanted into the endometrial lining of a woman's uterus.
- Morning sickness can occur in about seventy percent of all pregnant women and typically improves after the first trimester.
- Most miscarriages occur during this period.
- In the first trimester (the first three months) of pregnancy, most organogenesis, development of body organs occurs. The baby's heart starts beating at about four weeks

Second trimester

- Months 4 through 6 of the pregnancy are called the second trimester
- In the second trimester growth occurs. The baby is very active, and eventually these movements can be felt by the mother.
- The baby can hear by the fourth month, and when born recognizes not only mom's voice, but also dad's voice (if he's around)

Third trimester

- Final weight gain takes place, which is the most weight gain throughout the pregnancy. The fetus will be growing the most rapidly during this stage, gaining up to 28g per day.
- The baby tends to move less just because the uterus has become so crowded.
- Estrogen levels in the mother's blood reach their highest in the last weeks of pregnancy, and as this increases, it triggers the formation of oxytocin receptors in the uterus. Late in pregnancy, fetal cells produce increasing amounts of oxytocin. Oxytocin is a powerful smooth muscle stimulant, and due to the receptors which have formed in the uterus, causes uterine contractions.

Pregnancy test

A pregnancy test attempts to determine whether or not a woman is pregnant.

Pregnancy detection kits

It is either a strip or card impregnated with anti-HCG globulin

Most tests employ a monoclonal antibody, which is specific to the β-subunit of hCG (β-hCG) to ensure that tests do not make false positives by confusing hCG with LH and FSH.

Sample

Urine pregnancy test

It is done 5 to 7 days after late menstrual period

A first morning urine sample (that has accumulated in the bladder overnight) provides the most accurate test results.

Blood pregnancy test

It is done3 to 4 days after late menstrual period

> **Note:** *quantitative human chorionic gonadotropin (hCG) can be detected in blood before it is detectable in the urine. A blood test can confirm a pregnancy about 6 days after the fertilized egg implants into the uterus (even before a missed menstrual period).*

Procedure

Test strip

Either hold in the urine stream or dip into a sample of urine.

An area on the end of the test strip changes color if hCG is present, meaning that woman is pregnant.

Card device

Place several drops of serum into a well in the testing device

An area of the device changes color if hCG is present, meaning meaning that woman is pregnant.

Test accuracy

The accuracy of pregnancy tests varies because:

- The days of a woman's menstrual cycle and ovulation can vary each month.
- The sensitivity of the pregnancy test kit (usually Urine or serum test can detect at sensitivity of 25 mIU/ml)

Note: *While a few pregnancy tests may be sensitive enough to detect a pregnancy on the first day of a woman's missed period, most test kits are more accurate about a week after a missed period.*

False Negative Pregnancy Tests

- ***Early Detection Attempt***

  One reason for a false negative pregnancy test could be that you took the test too early. It is possible that although your ovum might be fertilized, meaning you may be pregnant, but your body has not started secreting enough Human Chorionic Gonadotropin (hCG) for the test to measure.

  Note: *faint positive side line an hour after the test: in this case the woman may be pregnant but hCG level may not be high enough in the test sample so another test must be done few days later*

- ***Using diluted urine***

  In early pregnancy, it is possible that drinking a lot of water and testing late in the day could affect the accuracy of the test. But this should only be a factor in the first few days after your missed menstrual period; after that, usually even a test later in the day should give a positive result.

  Note: *If you get a negative pregnancy test after having previously had a positive pregnancy test, you may be having a miscarriage -- especially if you are also having vaginal bleeding*

False Positive Pregnancy Test

- ***Improper testing***

Some tests will produce a faint positive test result if read after the instructed time. You should use a clock or a watch and record the time you perform the test and read results at the instructed time.

Note: *Most manufacturers suggest to read their test after 3 minutes and to NOT READ it after 10 minutes. Once you have a negative test after 3-5 minutes THROW OUT the test. Any positive test appearing only well after that time is inaccurate and cannot be considered positive.*

- ***Test interference***

Women receiving hCG therapy to treat a luteal phase defect or other fertility problem should consult their doctor when testing should be performed because it's possible to have a positive urine or blood pregnancy test for two to three weeks after the treatment and not be pregnant..

- ***Medical conditions***

Certain forms of cancer, such as choriocarcinoma (an extremely rare type of cancer usually found in the uterus) can cause an elevation in hCG levels as well as certain types of breast, or ovarian cancer leading to a false positive result.

General hematology

Blood

Blood is a specialized bodily fluid that delivers necessary substances to the body's cells — such as nutrients and oxygen — and transports waste products away from those same cells. In terms of anatomy and histology, blood is considered a specialized form of connective tissue

Functions

- Blood performs many important functions within the body including:
- Supply of oxygen to tissues (bound to hemoglobin, which is carried in red cells)
- Supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins (e.g., blood lipids)
- Removal of waste such as carbon dioxide, urea, and lactic acid
- Immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies
- Coagulation, which is one part of the body's self-repair mechanism
- Messenger functions, including the transport of hormones and the signaling of tissue damage
- Regulation of body pH (the normal pH of blood is in the range of 7.35 - 7.45)
- Regulation of core body temperature

Plasma

About 55% of whole blood is blood plasma, a fluid that is the blood's liquid medium, which by itself is straw-yellow in color.
The blood plasma volume totals of 2.7 – 3.0 liters in an average human.
It is essentially an aqueous solution containing 92% water, 8% blood plasma proteins (albumin , globulins and fibrinogen), and trace amounts of other materials such as glucose, mineral ions, amino acids, fatty acids and hormones .

Blood cells

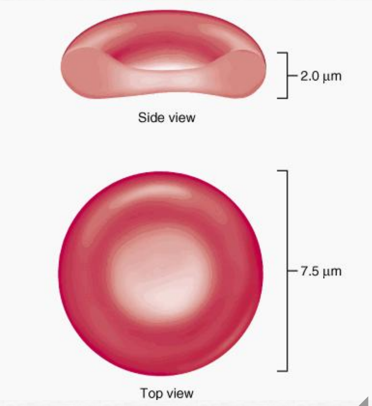
A blood cell is a cell of any type normally found in blood. In mammals, these fall into three general categories:

- ***red blood cells* — *Erythrocytes***
- ***white blood cells* — *Leukocytes***
- ***platelets* — *Thrombocytes***

Together, these three kinds of blood cells sum up for a total 45% of blood tissue by volume (and the remaining 55% is plasma). This is called the hematocrit.

1- Red blood cell

Red blood cells are also known as RBCs, red blood corpuscles. In humans, red blood cells develop in the bone marrow and live for about 120 days; they take the form of flexible biconcave disks that lack a cell nucleus and organelles and they cannot synthesize protein. The cells are filled with hemoglobin, a biomolecule that can bind to oxygen.



Function

- it is responsible for binding oxygen in the lung and transporting the bound oxygen throughout the body where it is used in aerobic metabolic pathways.

Hemoglobin

Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells. Hemoglobin transports oxygen from the lungs to the rest of the body where it releases the oxygen for cell use.

Hemoglobin Biosynthesis

Hemoglobin synthesis requires the coordinated production of heme and globin.

- ***Heme Synthesis***

  Heme is synthesized in a complex series of steps involving enzymes in the mitochondrion and in the cytosol of the cell. The first step in heme synthesis takes place in the mitochondrion, with the condensation of succinyl CoA and glycine by ALA synthase to form 5-aminolevulic acid (ALA). This molecule is transported to the cytosol where Heme biosynthesis occurs.

- ***Globin Synthesis***

  Globin protein parts are synthesized by ribosomes in the cytosol., four different globin chains are found. The peptide subunits are designated α, β, γ and δ which are arranged into the most commonly occurring functional hemoglobins.

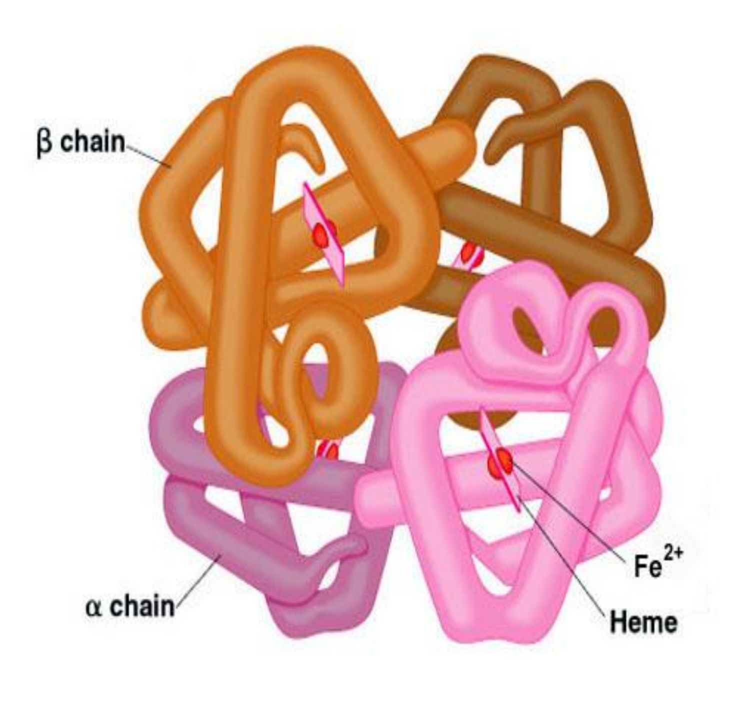
  Note: *Production of Hb continues in the cell throughout its early development from the proerythroblast to the reticulocyte in the bone marrow. At this point, the nucleus is lost in mammalian red blood cells. Even after the loss of the nucleus in mammals, residual ribosomal RNA allows further synthesis of Hb until the reticulocyte loses its RNA soon after entering the vasculature.*

Structure of Hemoglobin

the hemoglobin molecule is an assembly of four globin protein subunits (tetramer). Each subunit is composed of a protein chain tightly associated with a non-protein heme group.

The four polypeptide chains are bound to each other by salt bridges, hydrogen bonds, and hydrophobic interactions.

A heme group consists of an iron (Fe) ion (charged atom) held in a heterocyclic ring, known as a porphyrin. This porphyrin ring consists of four pyrrole molecules cyclically linked together with the iron ion bound in the center. The iron ion, which is the site of oxygen binding, coordinates with the four nitrogens in the center of the ring, which all lie in one plane. The iron is bound strongly to the globular protein via the imidazole ring of the histidine residue below the porphyrin ring.



Hemoglobin variants

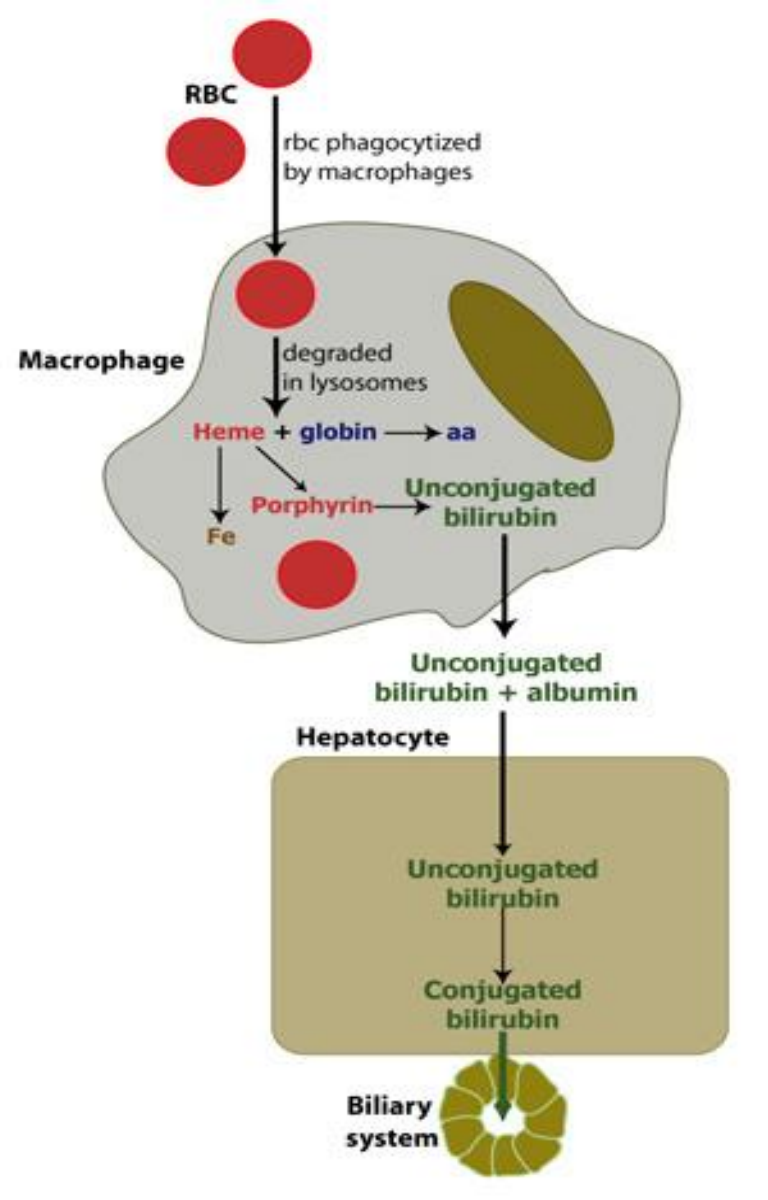
They are a part of the normal embryonic and fetal development, but may also be pathologic mutant forms of hemoglobin in a population, caused by variations in genetics.

- **Hemoglobin A (α2β2)** - The most common hemoglobin type with a normal amount over 95% consisting of two α and two β subunits.
- **Hemoglobin A2 (α2δ2)** - δ chain synthesis begins late in the third trimester and in adults, it has a normal range of 1.5-3.5%
- **Hemoglobin F (α2γ2)** - In adults Hemoglobin F is restricted to a limited population of red cells called F-cells. However, the level of Hb F can be elevated in persons with sickle-cell disease.
- **Hemoglobin H (β4)** - A variant form of hemoglobin, formed by a tetramer of β chains, which may be present in variants of α thalassemia.
- **Hemoglobin S (α2β^{S}2)** - A variant form of hemoglobin found in people with sickle cell disease. There is a variation in the β-chain gene, causing a change in the properties of hemoglobin, which results in sickling of red blood cells.

RBCs degradation

When red cells reach the end of their life due to aging or defects, they are broken down, the hemoglobin molecule is broken up:

- The globins are broken down to amino acids, which are then used for protein synthesis.
- The porphyrin ring of heme is oxidized by microsomal heme oxygenase, producing biliverdin and releasing the iron (Fe3+).
    - The iron can then be exported into plasma through iron channels, where it binds to apotransferrin forming transferrin and gets recycled or can be stored within cells as ferritin
    - Biliverdin is reduced by biliverdin reductase to unconjugated bilirubin (water insoluble). The unconjugated bilirubin is released into the plasma, where it binds to albumin and is taken up by hepatocytes.



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2- White blood cell

White blood cells (WBCs), or leukocytes, are cells of the immune system defending the body against both infectious disease and foreign materials.

Five different and diverse types of leukocytes exist, but they are all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. Leukocytes are found throughout the body, including the blood and lymphatic system.

Types

There are five different types of WBCs that the body uses to fight infections or other causes of injury. These types - ***neutrophils***, ***lymphocytes***, ***basophils***, ***eosinophils***, and ***monocytes*** - are present in relatively stable percentages that may temporarily shift higher or lower depending on what is going on in the body.

white blood cells are often characterized as granulocytes or agranulocytes:

- **Granulocytes (polymorphonuclear leukocytes):**
  Leukocytes characterized by the presence of differently staining granules in their cytoplasm when viewed under light microscopy. These granules are membrane-bound enzymes which primarily act in the digestion of endocytosed particles.
  There are three types of granulocytes: ***neutrophils***, ***basophils***, and ***eosinophils***, which are named according to their staining properties.

- **Agranulocytes (mononuclear leucocytes):**
  Leukocytes characterized by the apparent absence of granules in their cytoplasm. The cells include ***lymphocytes***, and ***monocytes***.

3- Platelet

thrombocytes, also called platelets, are small, irregularly-shaped anuclear cells (i.e. cells that do not have a nucleus containing DNA), 2-4 µm in diameter, which are derived from fragmentation of precursor megakaryocytes. The average lifespan of a platelet is between 8 and 12 days.

Function

The function of platelets is the maintenance of hemostasis. This is achieved primarily by the formation of thrombi, when damage to the endothelium of blood vessels occurs.

Haematopoiesis

Haematopoiesis is the formation of blood cellular components. All cellular blood components are derived from haematopoietic stem cells.

Locations

- ***Mesoblastic phase:*** In developing embryos, blood formation occurs in aggregates of blood cells in the yolk sac, called blood islands this occurs in the $3^{rd} - 4^{th}$ week.
- ***Hepatic phase:*** As development progresses, blood formation occurs in the spleen, liver and lymph nodes this occurs at the 6^{th} week of intrauterine life..
- ***Bone marrow or myeloid phase:*** When bone marrow develops, it eventually assumes the task of forming most of the blood cells for the entire organism this occurs at the 12^{th} week of pregnancy reaching full activity at 30^{th} w. with decline of liver sources that stop compeletly at birth.

Note: *Red marrow occupies whole skeletal system which gradually shrinks until the 18th years of age where the red marrow is restricted to the end of long bones, and flat bones where it is replaced by fatty marrow.*

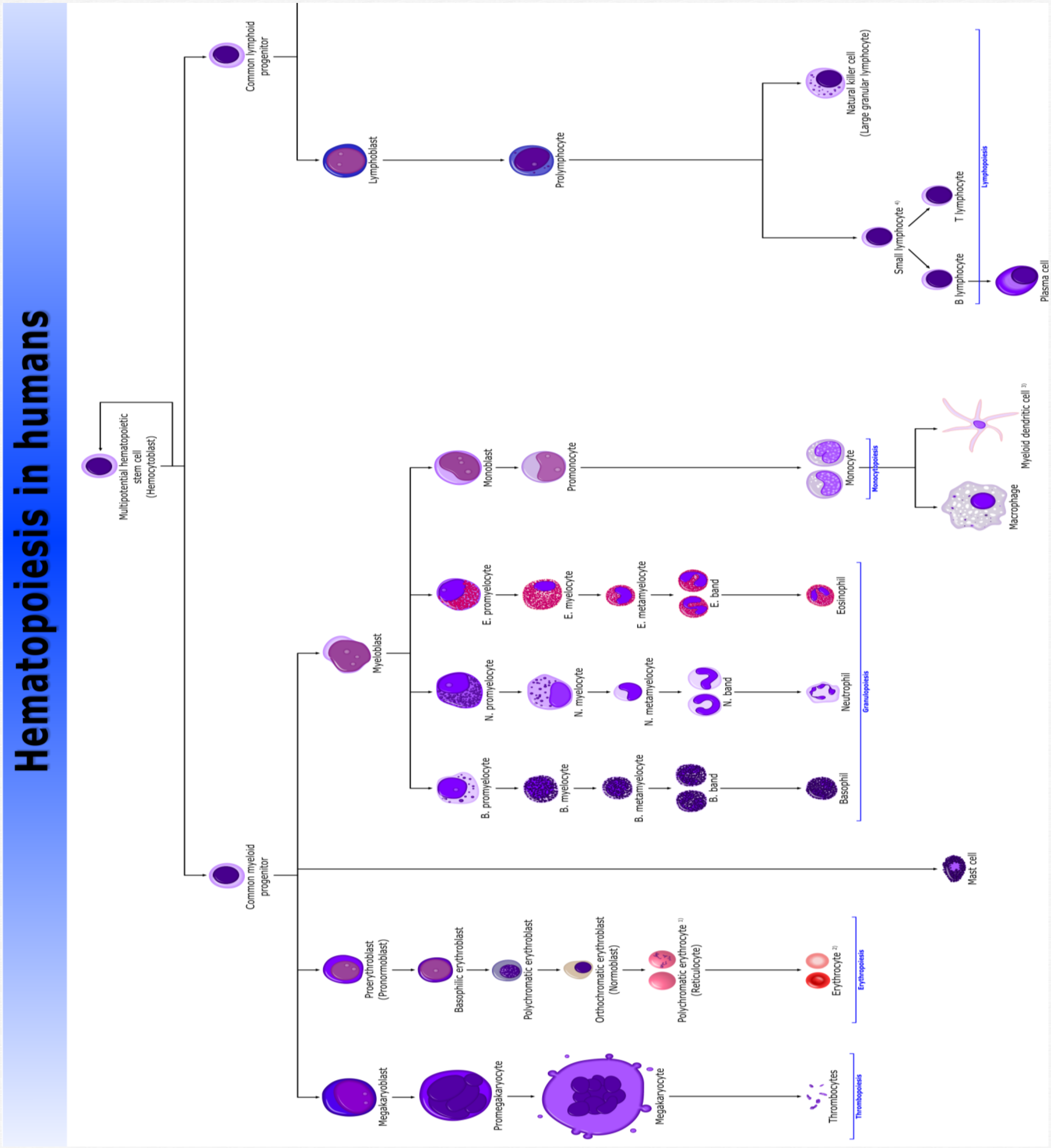
Note: *Sometimes under stress of life such as hemorrhage, the liver and splene revert to the embryonic state where a pool of more blood cells production is needed (extra-medullary hematopoiesis or myeloid metaplasia).*

<u>Steps in blood formations:</u>

All blood cells are derived form the undifferentiated primitive cells known as stem cells.

<u>Three steps</u> <u>are involved in formation in blood cells:</u>

- Multiplication of the developing cells which takes place by mitotic division.
- Maturation, may be defined as the progressive development of full characteristics both structural and functional.
- Release into the blood stream. The factors controlling the relase are not fully understood.



Complete Blood Picture (CBC)

A complete blood count (CBC), also known as full blood count (FBC) or full blood exam (FBE) or blood panel, is a test requested by a doctor that gives information about the cells in a patient's blood.

Why it is done?

A complete blood count may be done to:

- Evaluate the cause of certain symptoms such as fatigue, weakness, fever, bruising, or weight loss.
- Detect anemia.
- Determine the severity of blood loss.
- Diagnose polycythemia
- Diagnose an infection.
- Diagnose diseases of the blood, such as leukemia.
- Monitor the response to some types of drug or radiation treatment.
- Evaluate abnormal bleeding.

Sample

Whole blood collected on EDTA

What Affects the test

Factors that can interfere with your test and the accuracy of the results include:

- Prolonged use of a tourniquet while drawing the blood sample.
- Medications that can cause low platelet levels. These include steroids, some antibiotics, thiazide diuretics.
- Very high levels of triglycerides can cause falsely high hemoglobin values.
- Clotting of blood in the test tube.
- Clumping of platelets in the test tube. This can result in a decreased platelet count and occurs because of the substance used in the test tube.

Methods of blood count

Manual blood count

Hemacytometry

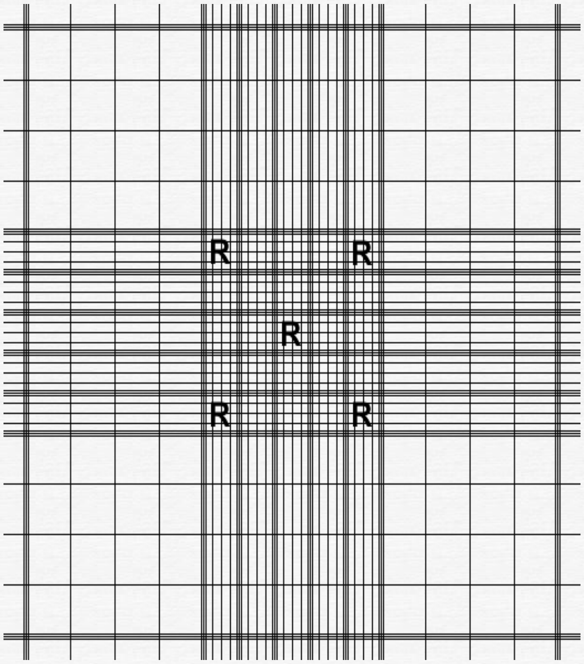
Hemacytometry means the use of the hemacytometer counting chamber to count blood cells (to count WBC, RBC, and Platelets).

Hemacytometer

Hemacytometer is a counting chamber device made of heavy glass with strict specifications

When viewing the hemacytometer from the top (figure below), it has 2 raised platforms surrounded by depressions on three sides, each raised platform has a ruled counting area marked off by precise lines etched into the glass. The raised areas and depression form H letter,

Hemacytometer Counting Areas



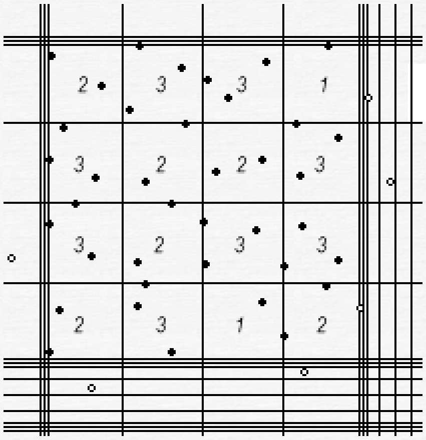
- Hemacytometer has 2 identical ruled counting areas, four corner squares and the center square
- The central square is further subdivided into 25 smaller squares and each smaller square is further subdivided into 12 smaller squares
- Each corner square is further subdivided into 12 smaller squares
- Platelets and RBCs are counted in the entire large center squares
- WBC cells are counted in the four corner squares

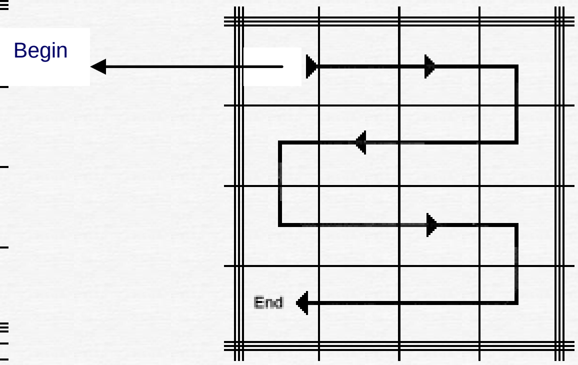
Using The Hemacytometer

- Clean cover slip slide (especially made for the hemacytometer).
- Position a clean, dust free, coverslip so it covers the ruled counting areas of a clean hemacytometer.
- Fill the hemacytometer with the fluid containing cells to be counted, by touching the tip of the capillary tube or micropipette tip to the point where the coverslip and raised platform meet on one side, the fluid will drawn under the coverslip and over the counting area by capillary action, this requires about 10 μl.
- The chamber must not be overfilled or underfilled, if accurate results are needed!.
- Place the hemacytometer on the microscope stage, so one of the ruled counting areas is aligned directly above the light source (condenser); rotate the low power objective (x10) into place and adjust your vision

The Counting Pattern

Either left to right or right to left counting pattern can be used but with the insurance that each cell is counted only once, to accomplish this, cells that touch the right boundary lines or the bottom boundary lines are not counted, because they will be counted with the other squares .





White blood cell (WBC) count

It is the count of the actual number of white blood cells per volume of blood.

Preparation solution (*Turkey's solution*):

3% glacial acetic acid (Weak acids will lyse red blood cells, and will darken WBC's to facilitate counting by the hemacytometer.)

> **Note:** *1 ml of Leishman stain can be added to the diluent to color the white blood cells, thus counting will be easier.*

Dilution factor:

1: 20 (i.e. 380 μl + 20 μl)

> **Note:** *Use a piece of cotton to wipe the outside of the pipette before inserting in the tube*

Method

- Mix the blood sample gently but thoroughly by inversion before preparation
- Pipette (380 μl) of diluting fluid into a tube.
- Pipette (20 μl) of well mixed blood and wipe the tip with cotton piece
- Put blood into the tube containing diluting fluid and mix well with the tip.
- Let the tube stand for 2-3 minutes to ensure complete RBC lyses, then mix well.
- Prepare the clean hemacytometer and cover it with the designed cover slip.
- Load one side of the hemacytometer with the aid of a micropipette
- Allow the hemacytometer to sit for several minutes to allow the WBC's to settle in the counting chamber, to avoid drying effect, place the loaded hemacytometer in a covered Petri dish with a moist gauze, until counting.
- Place the hemacytometer in the microscope stage.
- The WBC's are counted in the four corner large squares

> **Note:** *If the number of cells in a square varies from any other square by more than 9 cells, the count must be repeated, because this represents an uneven distribution of cells, which is may be caused by improper mixing of the dilution or improperly filled hemacytometer.*

Sources of Error:

- Contaminated diluting fluid
- Incorrect dilution
- Uneven distribution of WBC's
- Presence of clumped WBC's
- Unclean hemacytometer or cover slips
- Presence of air bubbles
- Incompletely filled hemacytometer
- Over flow
- Drying of the dilution in the hemacytometer

Calculations

Total WBC Count / cumm = no. of WBCs in 4 squares x 50

Results:

High leucocytes count is known as ***leucocytosis***

Low leucocytes count is known as ***leucopenia***

Red blood cell (RBC) count

It is the count of the actual number of red blood cells per volume of blood.

Preparation solution:
Isotonic saline (0.85% sodium chloride (NaCl) in distilled water)

Note: *saline cause slight crenation of RBCs and allow rouleaux formation*

Dilution factor:
1:200

Calculations

Total RBCs Count / cmm = no. of RBCs in 5 squares x 10,000

Results:
High erythrocytes count (and hemoglobin) is known as ***polycythemia***
Low erythrocytes count (and hemoglobin) is found in cases of ***anemia***

Hemoglobin concentration

It measures the amount of oxygen-carrying protein in the blood.

Reagent
Drabkin's solution contains the following:-

1. *Potassium Ferricyanide*
2. *Potassium Cyanide.*
3. *Non- ionic Detergent* Triton X-100
4. *Dihydrogen Potassium Phosphate.*

Working Drabkin's Solution Preparation:

Ready made commercial Drabkin's reagent is diluted with distilled water in the proportion 1:10.

Principle

- Well mixed EDTA anticoagulated blood is diluted in Drabkin's solution; non-ionic detergent will lyse the red cells to liberate hemoglobin, and to decrease the turbidity caused by red cell membrane fragments by dissolving them.
- Then, hemoglobin is oxidized and converted to methemoglobin (Hi) by potassium ferricyanide, this step is accelerated by the dihydrogen potassium phosphate, and requires approximately 3 minutes for total conversion. Potassium cyanide will provide cyanide ions to form cyanomethemoglobin (HiCN), which have a broad spectrum of absorption at 540 nm.

Method
5 ml Drabkin's solution + 20 ul whole blood
Mix well and incubate for 5 min at room temperature then read absorbance against reagent blank at **540 nm**

Calculations
Read hemoglobin concentration directly from the hemoglobin standard curve.

The platelet count

Platelets are the smallest formed elements in the blood, normally ranging in size from 2-4 microns. They are actually fragments of cytoplasm that have broken off platelet precursors. Platelets function in the coagulation of blood.

Preparation solution:
3.8 % ammonium oxalate

Dilution factor:
1:20

Method

- Carefully charge hemacytometer with diluted blood by gently squeezing sides of reservoir until properly filled.
- Place the hemacytometer in a moist chamber, e.g., covered Petri dish, bottom lined with moistened gauze squares, and allow to stand for 10 minutes for cells to settle.
- Using bright light or phase microscope, place hemacytometer on microscope stage. With low power objective, bring ruled area into focus.
- Switch to 40X objective, locate large center square, and bring into focus.
- Count platelets in all 5 small squares within the large, center square.

Platelets are difficult to count due to:

- small size - often hard to differentiate from bacteria and debris
- adhesiveness - affinity for adhering to glass
- aggregation - tendency to clump together

Sources of Error:

- Platelets count should be carried out within 2h of blood collection , delay cause clumbing and disintegration of platelets
- Specimens stored for more than 24 hours allow aggregation of platelets
- Light adjustment is critical. If the condenser is not lowered it will fade out the platelets.
- Bacteria and debris can be misinterpreted as platelets. This type of artifact is generally much more retractile than platelets.

Calculations

Total platelets Count / cumm = no. of platelets in 5 squares x 1,000

Results:
High platelets count is known as ***thrombocytosis***
Low platelets count is known as ***thrombocytopenia***

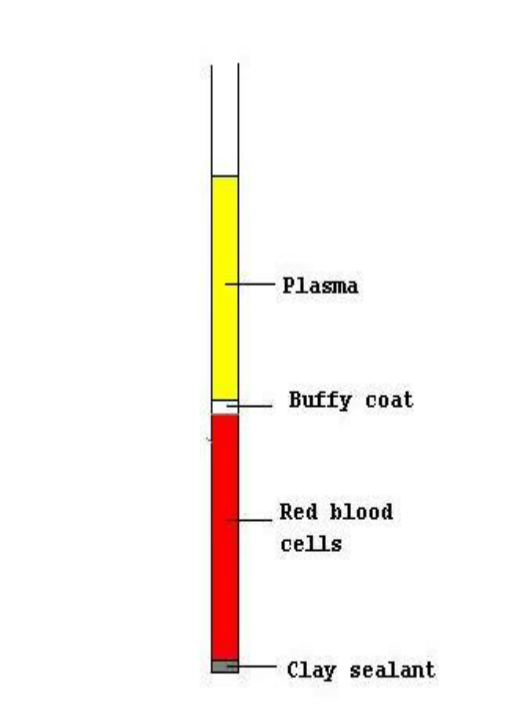
Red Blood Cell Indices

The red cell indices define the size and Hb content of the RBC and consist of the mean corpuscular volume (MCV), the mean corpuscular hemoglobin concentration (MCHC), and the mean corpuscular hemoglobin (MCH).
The RBC indices are used in differentiating anemias. When they are used together with an examination of the erythrocytes on the stained smear, a clear picture of RBC morphology may be ascertained.

Hematocrit

Hematocrit is the ratio of the total volume of RBC's to that of whole blood expressed as percentage (%) (Whole blood = total volume of cells + plasma).
For example, a hematocrit value of 40% means that there are 40 milliliters of red blood cells in 100 milliliters of blood.

Note: *The second synonym for hematocrit is PCV (Packed Cell Volume).*



Principle

The procedure is easy to perform, whole blood is centrifuged in a narrow tube (capillary tube), cellular elements will be separated from the plasma, after centrifugation blood will be separated into 3 layers :

(1) Bottom layer contains packed RBC's
(2) Middle layer contains WBC's and Platelets (Buffy coat)
(3) Upper plasma layer.

Method (microhaematocrate method)

- Fill the capillary tube with blood by capillary attraction.
- Seal with the clay the end of the capillary tube.
- Place and position the capillary tube in the centrifuge.
- Centrifuge for 15 minutes at 4000 g, so that additional centrifugation does not pack the red blood cells more.

Note: *Adequate centrifugation time and speed are important for accurate hematocrit because cells should be packed so that additional centrifugation does not alter or reduce HCT reading.*

Sources of Error:

- Inadequate mixing of the blood.
- Hemolysis of blood sample (due to improper collection, delay in processing) will cause erroneously decreased HCT.
- Failre to centrifuge long enough
- Excess EDETA cause decrease in PCV due to RBCs shrinkage
- Improper reading of the column lengths.
- Plasma trapping is still one of the causes of erroneously increased HCT results.

Note: *trapped plasma is usually 5% of PCV value so we can calculate true PCV as follow:*

True PCV = calculated PCV - 5% of PCV value

Calculations

Calculation of PCV

The height of the RBC column, and the total column should be measured with the aid of a ruler in cm and mm, then divide the RBC column height over the total column height (total height = RBC column + buffy coat + plasma column)

Calculation of Hb from HCT

Hb conc. = (Normal Hb X Calculated HCT) / Normal HCT

Mean corpuscular volume (MCV)

Mean Cell (Corpuscular) Volume, is the average volume of red cells. This parameter is useful in classifying anemia's into:

Microcytic, normocytic, and macrocytic.

MCV is calculated from the hematocrit (HCT), and the Red Blood Cells Count (RBC count).

MCV = (HCT / RBC) X 10

The results of MCV are expressed in femtoliters (fl).

> **Note:** *the automated hematology analyzers measure (not calculating) MCV, and then calculating the HCT from MCV and Total RBC count.*

Mean corpuscular hemoglobin (MCH)

Mean Cell Hemoglobin, is the hemoglobin content in the average red blood cell, or in other words, the average weight of hemoglobin per RBC.
It is calculated from the hemoglobin concentration (Hb), and the total RBC count.

MCH = (Hb / RBC) X 10

Results of MCH are expressed in picograms (pg)..

- **Macrocytic red cells** have higher MCH, because they are larger and contain more hemoglobin.
- **Microcytic red cells** have lower MCH, because they are smaller and contain less hemoglobin.

Mean corpuscular hemoglobin concentration (MCHC)

Mean Cell Hemoglobin Concentration, is the average hemoglobin concentration in 100 cc red blood cells. It indicates the average weight of hemoglobin as compared to the cell size. It correlates with the degree of hemoglobinization of the red cells on the peripheral blood film.
MCHC is calculated from the hematocrit and hemoglobin as follow:

MCHC = (Hb / HCT) X 100

Results of MCHC are expressed in percentage (%) or gm/dl.

- If results are within this range, it is said that red cells are Normochromic.
- If results are less than normal, red cells are said to be Hypochromic, which is seen in microcytic hypochromic anemias e.g. iron deficiency anemia.

Red cell distribution width (RDW)

Red cell distribution width (RDW) is a calculation of the variation in the size of your RBCs. In some anemias, such as pernicious anemia, the amount of variation (anisocytosis) in RBC size (along with variation in shape – poikilocytosis) causes an increase in the RDW.

Mean platelet volume (MPV)

Mean platelet volume (MPV) is a machine-calculated measurement of the average size of your platelets. New platelets are larger, and an increased MPV occurs when increased numbers of platelets are being produced. MPV gives your doctor information about platelet production in your bone marrow.

Automated CBC counters

Principle

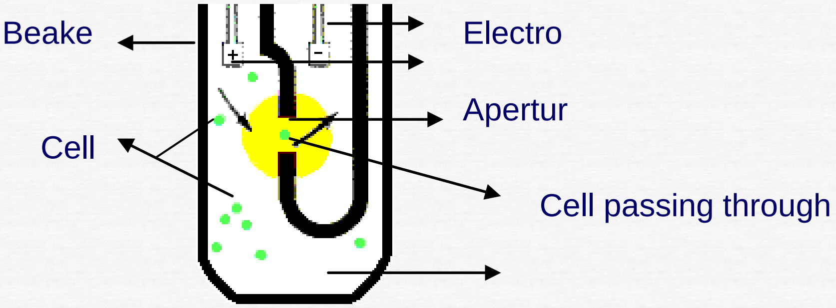
Automated cell counters quantify, classify, and describe cell populations using electrical **Impedance, Light Scattering** and optical detection techniques.

Electrical Impedance

These counters are based on the ***Coulter principle*** (The Coulter principle was named for its inventor, Wallace H. Coulter), i.e. electrical resistance principle, which depends on the fact that blood cells are nonconductive to electricity, so when they pass through an electrical field they will increase the electrical impedance (resistance).

This electrical resistance is manifested as a pulse, each pulse means a cell/a particle, sum of these pulses equals the total cell count.

when the suspended cells/particles passes through the aperture it will displace its own volume of isotonic electrolyte solution and increase the electrical resistance (impedance) because of their non-conductivity between the two electrodes which are located on each side of the aperture.



When each cell passes through the orifice it imposes resistance which is recorded as voltage pulse that corresponds to :

- the counting of the cell
- size of cell which is measured by the degree of resistance and is proportional to the volume of the cell

Light Scattering

Scattered light is a consequence of a light beam making contact with a cell, resulting in either reflected or refracted light reaching the detector. The pattern of light scattering is dependent on cell size and shape (***internal complexity***) giving relative measures of these cellular characteristics as cells flow through the beam.

Optical detection

It is utilized to gain a differential count of the populations of white cell types. A dilute suspension of cells are stained and passed through a flow cell, which passes cells one at a time through a capillary tube past a laser beam. The reflectance, and transmission of light from each cell is analyzed by software giving a numerical representation of the likely overall distribution of cell populations.

Advantages of the automated analyzers

Cell counting with these instruments is rapid, objective, statistically significant (8000 or more cells are counted), and not subject to the distributional bias of the manual count.

> **Note**: *MCV, here is considered as a measured parameter, not calculated parameter and HCT are calculated from RBCs and MCV as follow HCT = (MCV x RBCs) / 10*

Disadvantages of the automated analyzers

The automated hematology analyzers also may produce cell counts which are falsely increased or decreased. Some analyzers, particularly the impedance based counters may not be able to correctly distinguish tiny clumps of platelets. Platelet clumps may be misclassified as leukocytes or erythrocytes. Furthermore, large or unidentifiable atypical cells, toxic immature neutrophils, and markedly reactive lymphocytes can also be misclassified.

> **Note**: *peripheral blood smear examination provides information that cannot be obtained from automated cell counting*

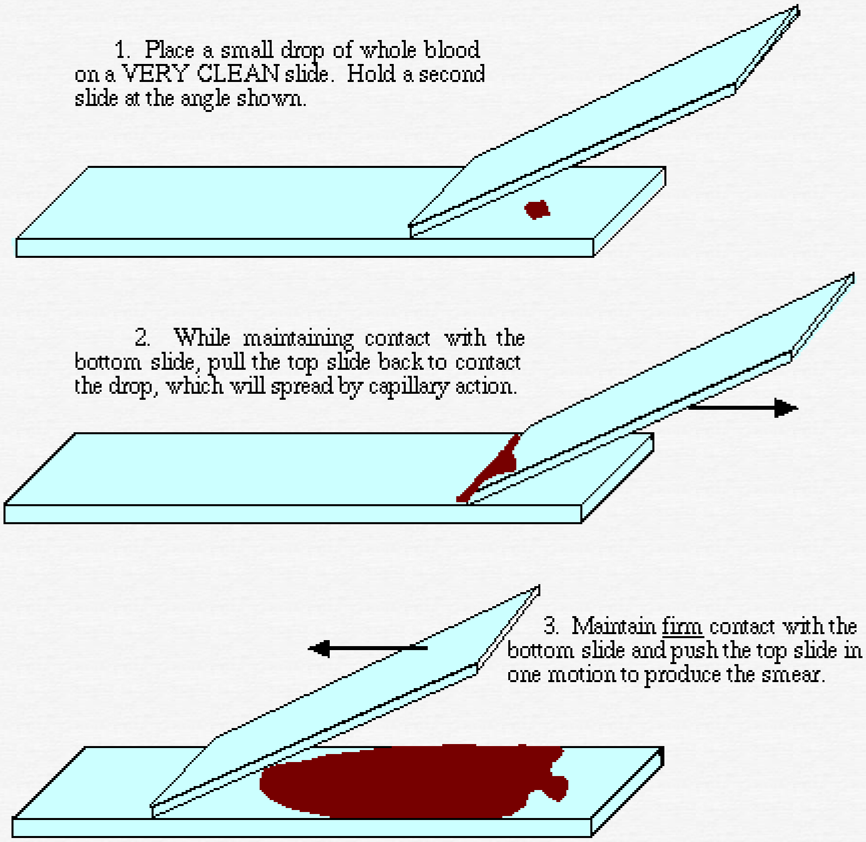
WBCs differential count

Importance

- Blood film enables us to evaluate WBC, RBC, and PLT morphology
- Allows us to perform differential WBC count
- Allows estimation of WBC and platelets counts from blood films

Preparation of Blood Films

- Use clean standard size glass slides, wiped from dust just immediately before use.
- Place a small drop of well mixed anticoagulated whole blood, in the slide.
- Immediately, without delay, with the aid of a second clean slide with uniform smooth edges (spreader slide), with a 30 –40 degrees angle, move back so blood drop will spread along the edge of the spreader slide, when this occurs, spread, or smear the film by a quick, uniform forward motion of the spreader.



Staining blood films

Blood films are stained so that morphology of blood cells become more easily viewed, identified, and evaluated. In addition, blood films may be examined for the presence of blood parasites (Malaria, Trypanosoma).

Leishman Stain Procedure:

- Let the films be air dried.
- Put the films on a staining trough rack.
- Flood the slides with the stain.

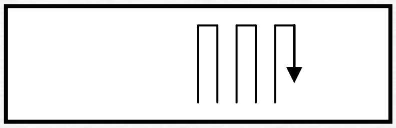
❖ After 2 minutes (or more, if the stain in newly prepared), add double volume of water, and blow to mix the stain with water, until a shiny layer is seen.
❖ After 5-7 minutes, wash with a stream of water.
❖ Wipe the back of the slides with cotton.
❖ Set the films in upright position on a filter paper to dry.

Sources Of Errors In Staining

❖ Stain Precipitate: May obscure cell details, and may cause confusion with inclusion bodies. Filter the stain before use.
❖ Improper stain timing may result in faded staining or altered colors:
  - Too long staining time causes too blue slides (overstaining).
  - Too short staining time causes too red slides.
❖ Excessive water on the slide or in the stain producing refractile edges on erythrocytes
❖ Forced drying may alter color intensities and/or distort cell morphology.

Examination of blood films

❖ Focus the film under x10 lens, and scan the film to check cell distribution.
❖ Add a drop of oil, and move to the x100 oil immersion lens.
❖ Choose a suitable area, where cells are evenly distributed without appreciable overlapping- the monolayer cell zone.
❖ Count the WBC's using tracking pattern.



❖ Each cell identified should be immediately tallied as:
  - ***Neutrophil- segmented.***
  - ***Neutrophil – band***
  - ***Lymphocyte***
  - ***Monocyte***
  - ***Eosinophil***
  - ***Basophil***
❖ Express the results as percentage for each cell subpopulation.

Cells	**Absolute normal**	**Relative normal**
Staff	**(0 - 300)**	**(0% - 3%)**
Segmented	**(2500 - 7000)**	**(40% - 60%)**
Lymphocytes	**(1700 - 3500)**	**(20% - 40%)**
Eosinophils	**(100 - 300)**	**(1% - 4%)**
Basophils	**(40 - 100)**	**(0.5% - 1%)**
Monocytes	**(200 - 600)**	**(2% - 8%)**

Absolute count

The absolute differential is derived by multiplying the percentage of each cell type by the WBC to get the number of each type of leukocyte /µl.
For example, if the WBC is 10,000 cells per microliter, the percentage of neutrophils is 10 and of bands is five, then the absolute neutrophil count is [(10 + 5) x 10,000]/100, or 1500 neutrophils per microliter of blood.

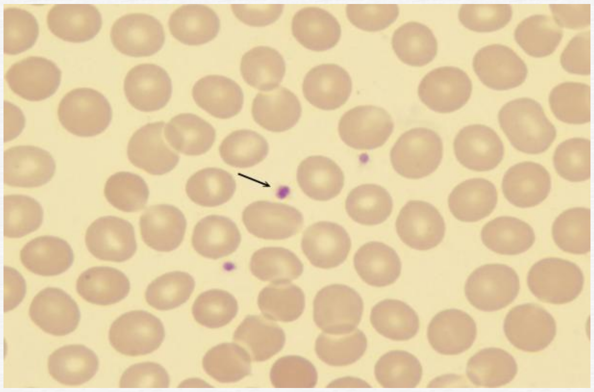
Blood Film WBC Quantitative Estimation

Total WBC count can be quantitatively estimated from blood films (under x 40 objective lens), also this estimation may be used for quality control purposes, and as delta check for manual and automated WBC counts, follow the table below for estimating WBC count in blood films:

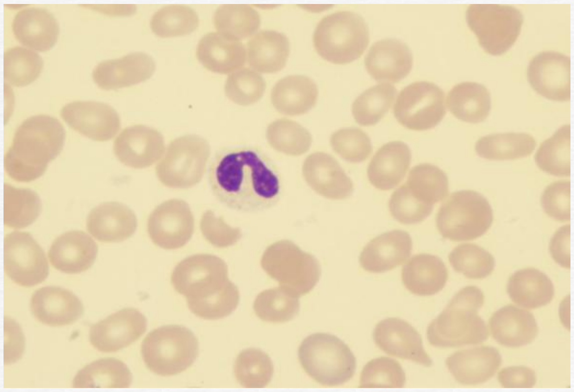
Number of WBC cells seen per x40 field	Estimated total WBC Count
2 – 4	4,000 – 6,000 /cumm
4 – 6	6,000 – 10,000 /cumm
6 – 10	10,000 – 13,000 /cumm
10 –13	13,000 – 20,000 /cumm

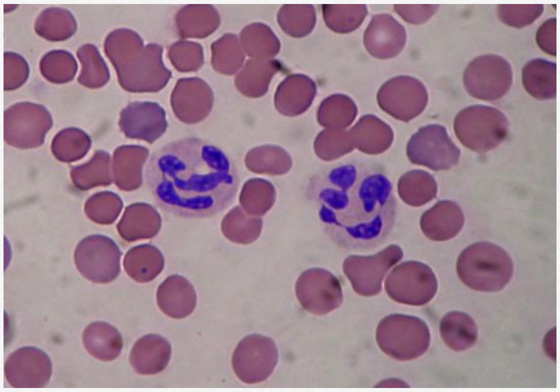
Blood Film Platelet Quantitative Estimation

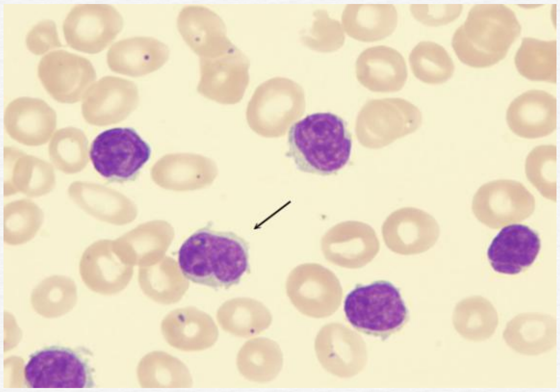
Platelets can be estimated quantitatively from blood films, each platelet seen under oil immersion lens approximately equals to 20,000 PLT/cumm. Normally, blood film from healthy individuals usually shows 7-22 platelets per oil immersion field.

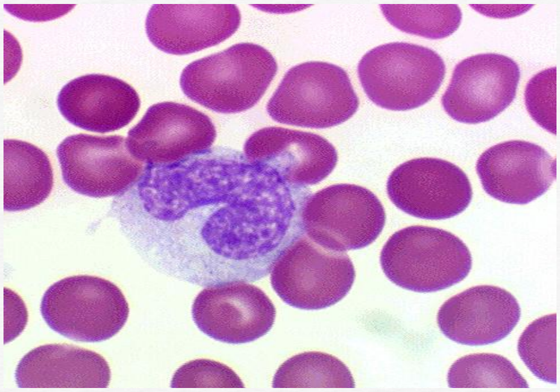


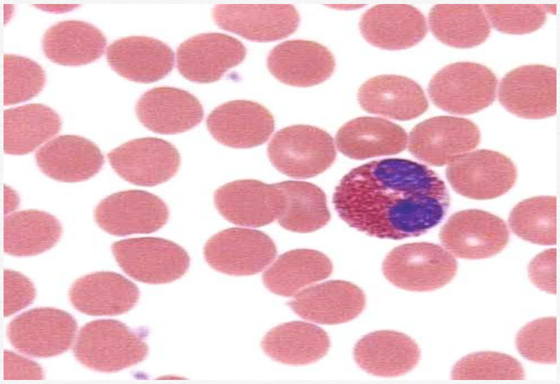
Note: *When platelet aggregates or clumps are present in the blood film, then platelet estimation would be absolutely unreliable.*

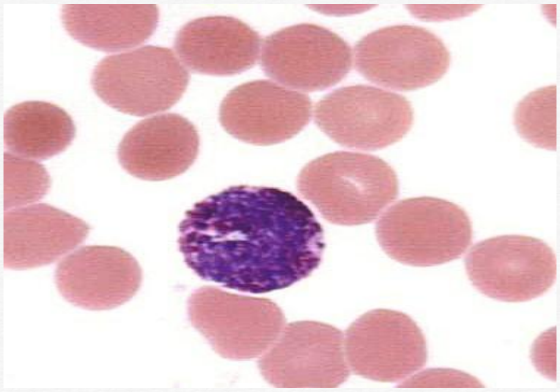












WBCs abnormalities in blood film

Immature WBC cells

Immature WBC cells (left shift) should be included in the differential. It is mainly seen in cases of leukemia. Examples include ***Blast cell*** , ***Promyelocyte*** , ***myelocyte***, ***neutrophilic juvenile***

hypersegmented neutrophils

Whereas normal neutrophils only contain three or four nuclear lobes, hypersegmented neutrophils contain five or more lobes. One of the earliest, notable changes in the peripheral blood in megaloblastic processes is the appearance of these hypersegmented neutrophils. Because of the short life-span of neutrophils, these abnormal hypersegmented neutrophils characteristically appear even before the onset of anemia

Toxic granulation

When a patient has an infection or a severe inflammatory disorder there is an increase in the neutrophil count and the neutrophils also show toxic granulation - coarse granules which are more deeply coloured than normal (It is thought to be the toxic precipitation of RNA) .

> **Note:** *It should be noted that 'toxic granulation' also occurs in normal pregnancy as well as in infection and inflammation.*

Reactive lymphocytes

Atypical, or reactive, lymphocytes are lymphocytes that, as a result of antigen stimulation, have become quite large, sometimes more than 30 µm in diameter. The cells vary greatly in size and shape. The nucleus is less clumped than that of the normal lymphocyte. Causes of reactive lymphocytosis may be: cytomegalovirus, drugs, Epstein-Barr virus (infectious mononucleosis), syphilis, toxoplasmosis, vaccination, auto-immune disorders such as rheumatoid arthritis and viral hepatitis.

RBCs abnormalities in blood film

Anisocytosis

Anisocytosis means that the red cells are of unequal size. It is a feature of many anemias, and other blood conditions, and does not have much diagnostic value. The RDW is useful in the differential diagnosis of microcytic anemia. Most cases of iron deficiency have a raised RDW, and most cases of thalassemia trait have a normal RDW.

Poikilocytes

Abnormal variation in shape. Poikilocytosis refers to the presence of poikilocytes in the blood. Poikilocytes are abnormally shaped red blood cells as seen on a blood film
It may be found in any severe anemia (eg, megaloblastic, iron-deficiency, hemolytic)

Microcytes

Small cells, (MCV <80 fL) It may be found in Iron-deficiency and thalassemia, and vitamin B6 deficiency

Macrocytes

Large cells (MCV >100 fL) It may be found in Megaloblastic anemia, alcoholism, liver disease, and hemolytic anemia

Hypochromia

Pale cells with decreased concentration of hemoglobin (MCHC <30 g/dL). It is seen in Severe iron-deficiency and, thalassemia

- **Spherocytes**

Spherical cells without pale centers. It is found in Hereditary spherocytosis, and Coombs'-positive hemolytic anemia

- **Sickle cells**

Sickle cells are red blood cells that have become crescent shaped. When a person with sickle cell anemia is exposed to dehydration, infection, or low oxygen supply, their fragile red blood cells form liquid crystals and assume a crescent shape causing red cell destruction and thickening of the blood. Since the life span of the red blood cell is shortened, there is a temporary depression of red cell production in the bone marrow, and a subsequent fall in hemoglobin

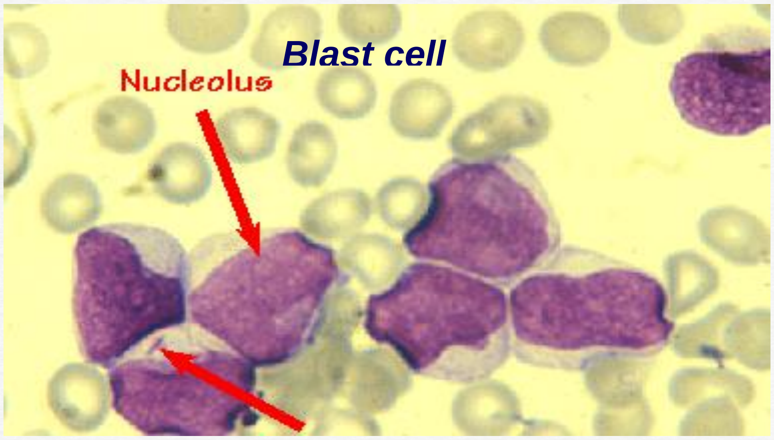
- **Target cells**

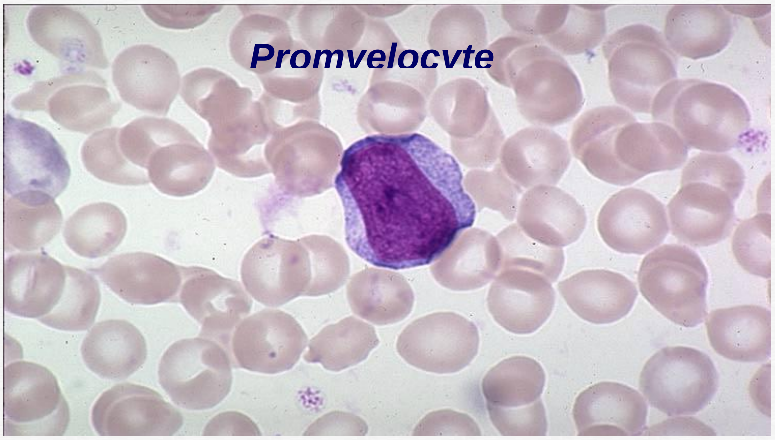
Cells with a dark center and periphery and a clear ring in between. It is seen in Liver disease, thalassemia, iron-deficiency anemia, hemoglobinopathies
Howell-Jolly bodies

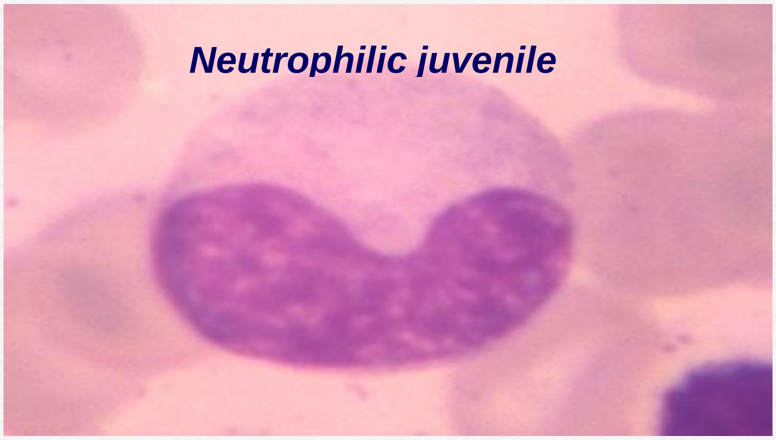
- **Howell-Jolly bodies**

Howell-Jolly bodies are spherical blue-black inclusions of red blood cells seen on Wright-stained smears. They are nuclear fragments of condensed DNA, 1 to 2 µm in diameter, normally removed by the spleen. They are seen in severe hemolytic anemias, in patients with dysfunctional spleens or after splenectomy.

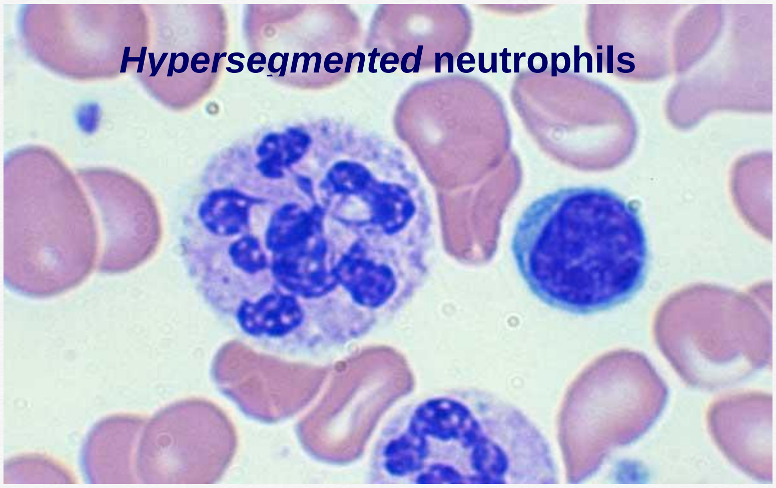
Immature leucocytes

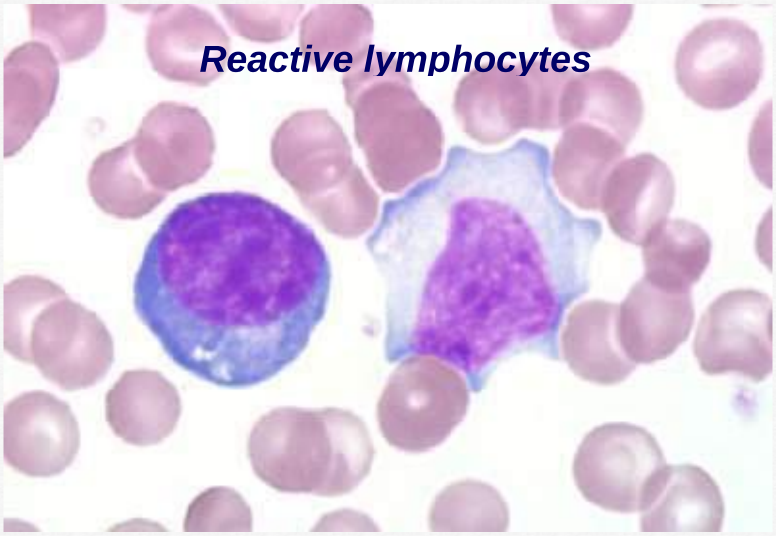




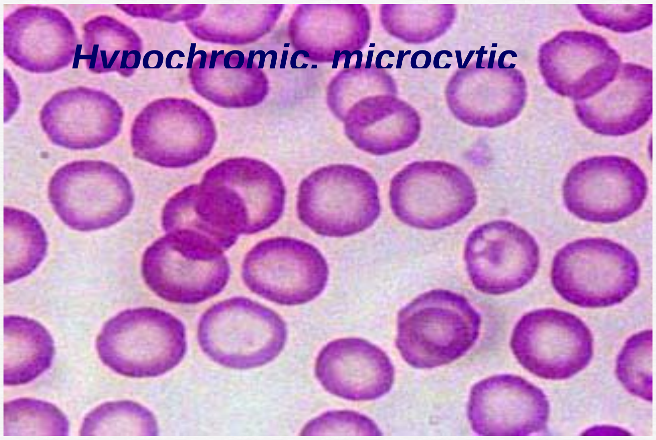


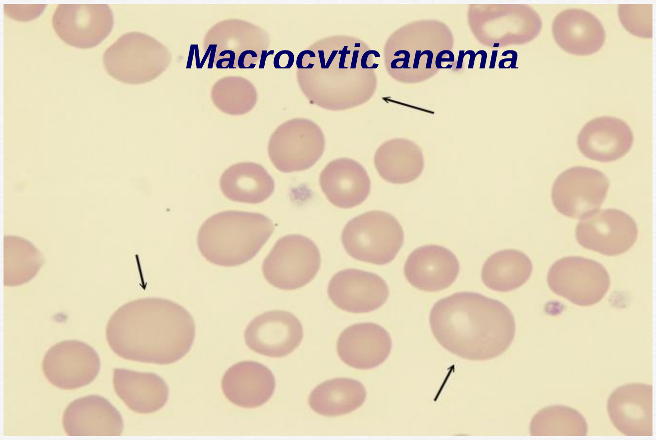
Abnormal leucocytes

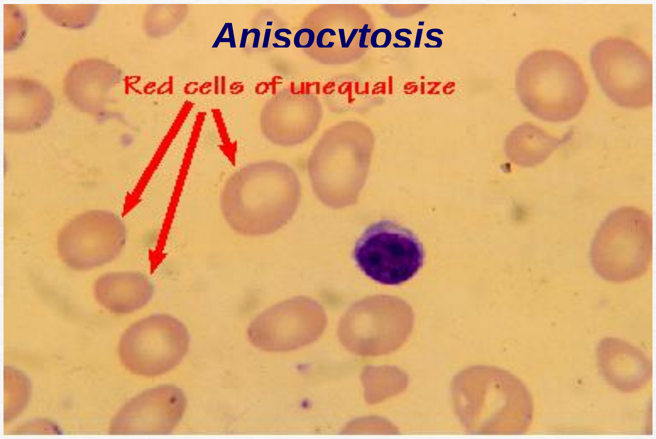


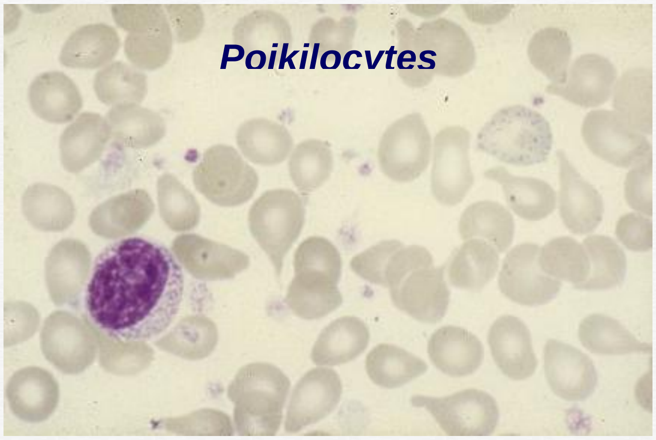


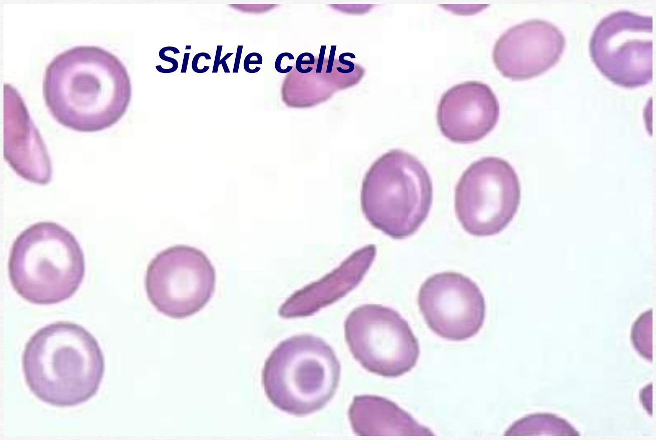
Abnormal Erythrocytes

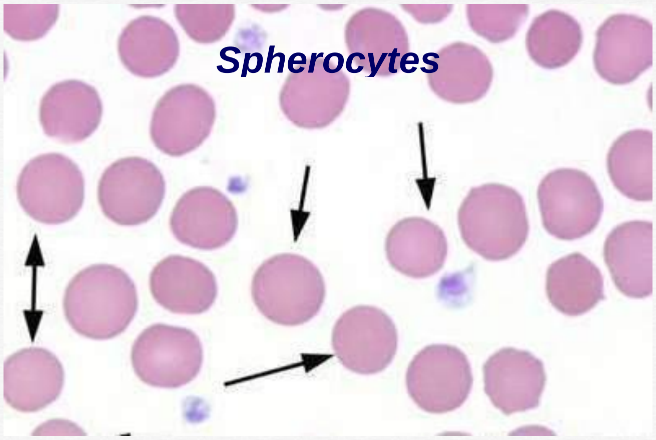


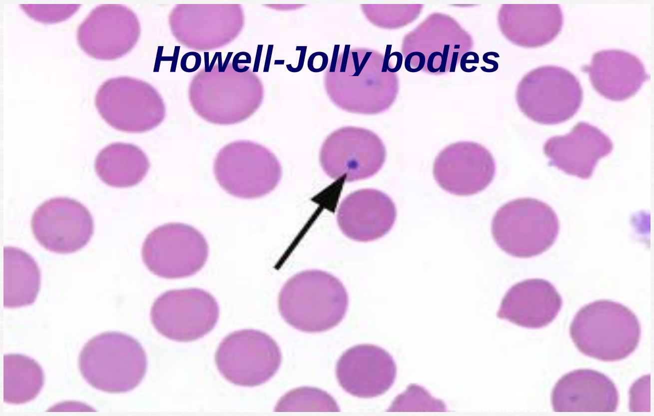


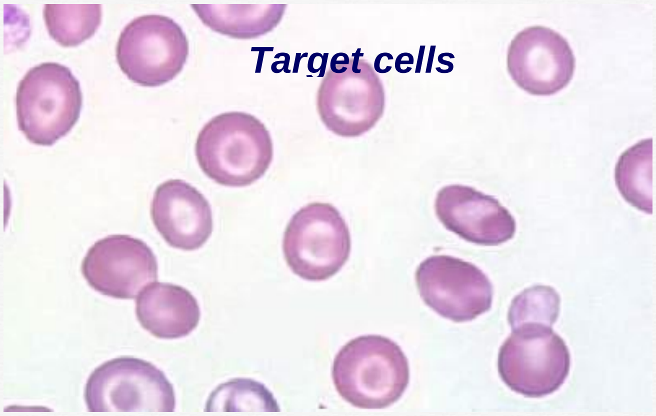












Interpretation of CBC results

RBCs abnormalities

Anemia

Anemia is a decrease in normal number of red blood cells (RBCs) or less than the normal quantity of hemoglobin in the blood.

What Are the Symptoms?

The symptoms of anemia vary according to the type of anemia, the underlying cause, and any underlying health problems.

The body also has a remarkable ability to compensate for early anemia. If your anemia is mild or developed over a long period of time, you may not notice any symptoms.

Symptoms common to many types of anemia include the following:

- Easy fatigue and loss of energy
- Unusually rapid heart beat, particularly with exercise
- Shortness of breath and headache, particularly with exercise
- Difficulty concentrating
- Dizziness
- Pale skin
- Leg cramps
- Insomnia

Classification of anemia

Etiological classification

The three main classes of anemia include:

Excessive blood lose

- Anemia of acute hemorrhage
- Anemia of chronic hemorrhage
    - Gastrointestinal conditions such as ulcers, hemorrhoids, gastritis (inflammation of the stomach) and cancer
    - Use of nonsteroidal anti-inflammatory drugs (NSAIDS) such as aspirin
    - Menstruation, especially if menstrual bleeding is excessive.

Excessive blood cell destruction

When red blood cells are fragile and cannot withstand the routine stress of the circulatory system, they may rupture prematurely, causing hemolytic anemia. Hemolytic anemia can be present at birth or develop later.

Known causes of hemolytic anemia may include any of the following:

- sickle cell anemia
- thalassemia
- hereditary spherocytosis
- G6PD deficiency anemia
- enlarged spleen (splenomegaly) can trap red blood cells and destroy them before their circulating time is up

Deficient red blood cell production

The body may produce too few blood cells or the blood cells may not work properly. In either case, anemia can result.

Conditions associated with these causes of anemia include the following:

- ❖ Iron deficiency anemia
- ❖ Pernicious anemia
- ❖ Aplastic anemia
- ❖ Advanced kidney disease

Morphological classification

The first job in working up a patient with anemia is to place the case in one of three major cytometric categories:

Normochromic, normocytic anemia (normal MCHC, normal MCV).

- ❖ Anemia of chronic disease as advanced kidney disease , liver disease , malignancy , sever inflammations and infection
- ❖ sickle cell anemia
- ❖ hereditary spherocytosis
- ❖ G6PD deficiency anemia
- ❖ anemia of acute hemorrhage
- ❖ Aplastic anemia
- ❖ anemia during pregnancy

Hypochromic, microcytic anemia (low MCHC, low MCV).

- ❖ iron deficiency anemia
- ❖ thalassemias

Normochromic, macrocytic anemia (normal MCHC, high MCV).

- ❖ Pernicious anemia
- ❖ Folate deficiency anemia

Common types of anemia

sickle cell anemia

- Sickle-cell disease (SCD), or sickle-cell anaemia , is a genetic blood disorder characterized by red blood cells that assume an abnormal, rigid, sickle shape. Sickling decreases the cells' flexibility and results in a risk of various complications. The sickling occurs because of a mutation in the haemoglobin gene

  (The sickle-cell disease occurs when the seventh amino acid, glutamic acid, is replaced by valine to change its structure and function).
- In people heterozygous for HgbS (carriers of sickling haemoglobin), the polymerisation problems are minor, because the normal allele is able to produce over 50% of the haemoglobin. In people homozygous for HgbS, the presence of long-chain polymers of HbS distort the shape of the red blood cell from a smooth donut-like shape to ragged and full of spikes, making it fragile and susceptible to breaking within capillaries.
- Carriers have symptoms only if they are deprived of oxygen (for example, while climbing a mountain) or while severely dehydrated.

Thalassemia

- Thalassemia is an inherited autosomal recessive blood disease. In thalassemia, the genetic defect results in reduced rate of synthesis of one of the globin chains that make up hemoglobin (underproduction of normal globin proteins, often through mutations in regulatory genes). Reduced synthesis of one of the globin chains can cause the formation of abnormal hemoglobin molecules, thus causing anemia, the characteristic presenting symptom of the thalassemias.

Thalassemia is a quantitative problem of too few globins synthesized, whereas sickle-cell anemia (a hemoglobinopathy) is a qualitative problem of synthesis of an incorrectly functioning globin.

- The thalassemias are classified according to which chain of the hemoglobin molecule is affected. In α thalassemias, production of the α globin chain is affected, while in β thalassemia production of the β globin chain is affected.

Hereditary spherocytosis

- Hereditary spherocytosis is a relatively common disorder characterized by red blood cells that are intrinsically defective because of their sphere-like shape.

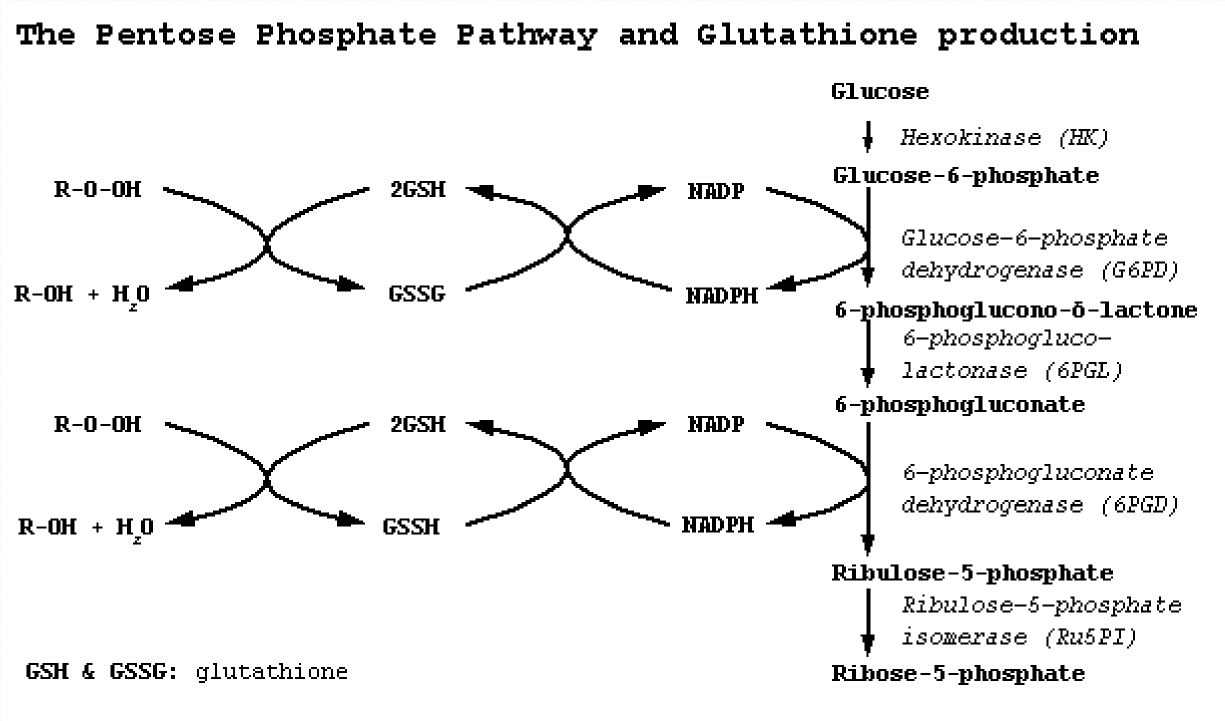
These cells have increased osmotic fragility -- they are more fragile than normal.

- Hereditary spherocytosis may be the result of an autosome dominant transmitted detect in red cell structural proteins. It is associated with a compensated or uncompensated hemolytic state, which is relieved by splenectomy. Most hereditary hemolytic anemia's involve mutations of membrane structural proteins, the majority ode for abnormal spectrin molecules.

G6PD deficiency anemia

- Glucose-6-phosphate dehydrogenase deficiency is an X-linked recessive hereditary disease characterized by abnormally low levels of glucose-6-phosphate dehydrogenase (G6PD), a metabolic enzyme involved in the pentose phosphate pathway, especially important in red blood cell metabolism.
- Individuals with the disease may exhibit hemolytic anemia in response to a number of causes, most commonly infection or exposure to certain medications or chemicals.

G6PD deficiency is closely linked to favism, a disorder characterized by a hemolytic reaction to consumption of broad beans.



Iron deficiency anemia

Iron deficiency anemia is a common type of anemia, and is known as sideropenic anemia. It is the most common cause of microcytic anemia.

Cause

- The principal cause of iron deficiency anemia in premenopausal women is blood lost during menses.
- Iron deficiency anemia can be caused by parasitic infections, such as hookworms. Intestinal bleeding caused by hookworms can lead to fecal blood loss and heme/iron deficiency.
- 60% of patients with iron deficiency anemia may have underlying gastrointestinal disorders leading to chronic blood loss.

The diagnosis of iron deficiency anemia will be suggested by a low serum ferritin, a low serum iron level, an elevated serum transferrin and a high total iron binding capacity (TIBC).

Pernicious anemia

Pernicious anemia (also known as Biermer's anemia, Addison's anemia) is one of many types of the larger family of megaloblastic anemias. It is caused by loss of gastric parietal cells, and subsequent inability to absorb vitamin B12.

Usually seated in an atrophic gastritis, the autoimmune destruction of gastric parietal cells leads to a lack of intrinsic factor. Since the absorption from the gut of normal dietary amounts of vitamin B12 is dependent on intrinsic factor, the loss of intrinsic factor leads to vitamin B12 deficiency.

Aplastic anemia

Aplastic anemia is a condition where bone marrow does not produce sufficient new cells to replenish blood cells. The condition, per its name, involves both aplasia and anemia. Typically, anemia refers to low red blood cell counts, but aplastic anemia patients have lower counts of all three blood cell types: red blood cells, white blood cells, and platelets, termed pancytopenia.

Causes

- In many cases, the etiology is considered to be idiopathic (cannot be determined), but one known cause is an autoimmune disorder in which white blood cells attack the bone marrow.
- Aplastic anemia is also sometimes associated with exposure to toxins such as benzene, or with the use of certain drugs, including chloramphenicol.
- Exposure to ionizing radiation from radioactive materials or radiation-producing devices is also associated with the development of aplastic anemia.

Polycythemia

Polycythemia (or polycythaemia) is a condition in which there is an increase in the proportion of blood volume that is occupied by red blood cells.

It can be due to an increase in the mass of red blood cells ("absolute polycythemia") or to a decrease in the volume of plasma ("relative polycythemia").

Absolute polycythemia

The overproduction of red blood cells may be due to:

Primary polycythemia (Polycythemia vera)

Primary polycythemia, often called polycythemia vera (PCV), polycythemia rubra vera (PRV), occurs when excess red blood cells are produced as a result of an abnormality of the bone marrow.

Polycythemia vera is classified as a myeloproliferative disease.

Criteria of polycythemia rubera vera:

- A hallmark of polycythemia is an elevated hematocrit, with Hct > 55% seen in 83% of cases.
- Leukocytosis (> 12.000 / mm3)
- Thrombocytosis (> 400.000 / mm3)
- Abnormal marrow karyotyping

Secondary polycythemia

Secondary polycythemia is caused by either natural or artificial increases in the production of erythropoietin, hence an increased production of erythrocytes.
Secondary polycythemia resolves when the underlying cause is treated.
Causes of secondary polycythemia include:

- Appropriate bone marrow response to physiologic conditions such as high altitude
- Renal or liver tumors
- Smoking
- Heart or lung diseases that result in hypoxia.

Relative polycythemia

Relative polycythemia is an apparent rise of the erythrocyte level in the blood; however, the underlying cause is reduced blood plasma. Relative polycythemia is often caused by loss of body fluids, such as through burns, dehydration and stress.

WBCs abnormalities

Leucocytosis

Leucocytosis: Increase in the number of circulating leucocytes more than 11 x 10^3 /cmm.
Leukocytosis is a raised white blood cell count (the leukocyte count) above the normal range. This increase in leukocytes (primarily neutrophils) is usually accompanied by a **"left shift"** in the ratio of immature to mature neutrophils. The increase in immature leukocytes increases due to proliferation and release of granulocyte and monocyte precursors in the bone marrow which is stimulated by several products of inflammation.

> **Note:** *A leukocyte count above 25 to 30 is termed a* leukemoid reaction*, which is the reaction of a healthy bone marrow to extreme stress, trauma, or infection. (It is different from leukemia, in which immature blood cells are present in peripheral blood.)*

Causes

Leukocytosis can be subcategorized by the type of white blood cell that is increased in number.

- Leukocytosis in which neutrophil is elevated is ***neutrophilia***.
- leukocytosis in which eosinophil count is elevated is ***eosinophilia***.
- leukocytosis in which basophile count is elevated is ***basophilia***.
- leukocytosis in which lymphocyte count is elevated is ***lymphocytosis***;
- leukocytosis in which monocyte count is elevated is ***monocytosis***;

Neutrophilia

Neutrophilia (or neutrophil leukocytosis) is a condition where a person has a high number of neutrophil granulocytes in their blood.

Causes

- Neutrophils are the primary white blood cells that respond to a bacterial infection, so the most common cause of neutrophilia is a bacterial infection.

- Neutrophils are also increased in any acute inflammation, so will be raised after a heart attack or other infarct.
- A neutrophilia might also be the result of a malignancy, Chronic myeloid leukemia (CML)

Physiologic conditions such as stress, excitement, fear, and exercise temporarily cause increased WBCs. Also crying babies have neutrophilia.

Lymphocytosis

Lymphocytosis is an increase in the number or proportion of lymphocytes in the blood

Causes

Causes of absolute lymphocytosis include:

- acute viral infections, such as viral hepatitis
- some protozoal infections, such as toxoplasmosis
- chronic intracellular bacterial infections such as tuberculosis or brucellosis
- Acute Lymphoblastic Leukemia

Causes of relative lymphocytosis include:

- age less than 2 years
- acute viral infections
- splenomegaly with splenic sequestration of granulocytes.

Eosinophilia

Eosinophilia is the state of having a high concentration of eosinophils (eosinophil granulocytes) in the blood.

Causes

- Diseases that feature eosinophilia:
- Parasitic infections (intestinal helminthiasis)
- Allergic disorders
- Some forms of chronic myeloid leukaemia
- Hodgkin's lymphoma

Basophilia

Basophilia: Ulcerative Colitis , Myeloproliferative Diseases,
Basophilia is a condition where the basophil quantity is abnormally elevated.

Causes

- Basophilia is a common feature of myeloproliferative disorders and particularly prominent in chronic granulocytic leukaemia
- Also increase in cases of allergy
- Chronic ulcerative colitis

Monocytosis

Monocytosis is an increase in the number of circulating monocytes

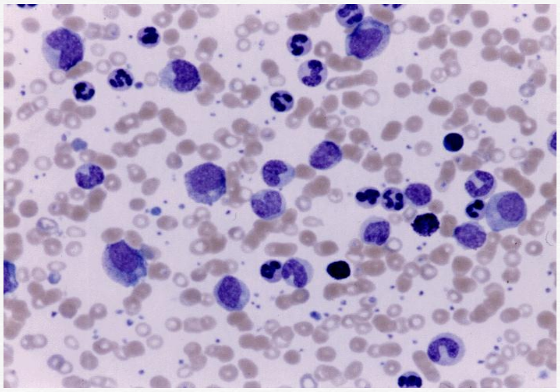
Causes

Monocytosis often occurs during chronic inflammation. Diseases that produce this state:

- Infections: tuberculosis, brucellosis, syphilis, infectious mononucleosis and other viral infections and many protozoal infections (e.g. kala azar, malaria)
- Autoimmune diseases: systemic lupus erythematosus, rheumatoid arthritis and inflammatory bowel disease.
- Malignancies: Hodgkin's disease and certain leukaemias, such as monocytic leukemia.

Leukemoid Reaction (LR):

A leukemoid reaction (LR) is a hematological disorder, defined by a leukocyte count greater than 50,000 cells/mcL. LR is characterized by a significant increase in mature neutrophils in the peripheral blood and a differential count showing left shift. LR is associated with infections.



Leucopenia

Leukopenia (also known as leukocytopenia) is a decrease in the number of white blood cells (leukocytes) found in the blood, which places individuals at increased risk of infection.

Causes

Leukopenia can be subcategorized by the type of white blood cell that is increased in number.

- Leukopenia in which neutrophil is decreased is ***neutropenia***.
- Leukopenia in which eosinophil count is decreased is ***Eosinopenia***.
- Leukopenia in which basophile count is decreased is ***Basopenia***.
- Leukopenia in which lymphocyte count is decreased is ***Lymphocytopenia***;

Neutropenia

is a sub-type of leukopenia that refers to a decrease in the number of circulating neutrophil granulocytes, the most abundant white blood cells. The terms leukopenia and neutropenia may occasionally be used interchangeably, as the neutrophil count is the most important indicator of infection risk.

Classification

There are three general guidelines used to classify the severity of neutropenia based on the absolute neutrophil count (ANC)

- Mild neutropenia (1000 <= ANC < 1500) — minimal risk of infection
- Moderate neutropenia (500 <= ANC < 1000) — moderate risk of infection
- Severe neutropenia (ANC < 500) — severe risk of infection.

Causes

Causes can be divided into the following groups:

Decreased production in the bone marrow:

- Aplastic anemia
- radiation
- Vitamin B12 or folate deficiency (Megaloblastic anemia).
- Drug induced: anti-inflammatory, anti-TB, anti bacterial (cloramphinecol and sulphonamides).
- bacterial infections as brucellosis and TB.

Increased leucocytes destruction:

- autoimmune neutropenia (SLE).
- chemotherapy treatments, such as for cancer and autoimmune diseases (such as methotrexate)
- Sequestration as in hemodialysis

Lymphocytopenia

Lymphocytopenia, or lymphopenia, is the condition of having an abnormally low level of lymphocytes in the blood.

Causes

- The most common cause of temporary lymphocytopenia is a recent infection, such as the common cold.
- Lymphocytopenia, is associated with Acquired immune deficiency syndrome (AIDS).
- Intense or prolonged physical exercise (due to cortisol release), rheumatoid arthritis, (caused by corticosteroid therapy).
- Lymphocytopenia is a frequent, temporary result from many types of chemotherapy, such as with cytotoxic agents or immunosuppressive drugs.

Eosinopenia

Eosinopenia is a form of agranulocytosis where the number of eosinophil granulocyte is lower than expected.

Causes

- It can be induced by the use of steroids.

Basopenia

Basopenia (or basocytopenia) is a form of agranulocytosis associated with a deficiency of basophils

Causes

- One cause is urticaria.

Note: *it is difficult to detect without flow cytometry, because normal levels are so low.*

Leukemia

Leukemia is a cancer of the blood or bone marrow and is characterized by an abnormal proliferation of blood cells, usually white blood cells (leukocytes).

Classification

Leukemia is clinically and pathologically subdivided into several large groups. The first division is between its acute and chronic forms:

- **Acute leukemia** is characterized by the rapid increase of immature blood cells. This crowding makes the bone marrow unable to produce healthy blood cells. Immediate treatment is required in acute leukemia due to the rapid progression and accumulation of the malignant cells
- **Chronic leukemia** is distinguished by the excessive build up of relatively mature, but still abnormal, white blood cells. Typically taking months or years to progress, the cells are produced at a much higher rate than normal cells, resulting in many abnormal white blood cells in the blood. Whereas acute leukemia must be treated immediately, chronic forms are sometimes monitored for some time before treatment to ensure maximum effectiveness of therapy.

Major types of leukemia

- ***Acute lymphocytic leukemia* (ALL)** is the most common type of leukemia in young children. This disease also affects adults, especially those age 65 and older. Standard treatments involve chemotherapy and radiation. The survival rates vary by age: 85% in children and 50% in adults.

- ***Chronic lymphocytic leukemia (CLL)*** most often affects adults over the age of 55. It sometimes occurs in younger adults, but it almost never affects children. Two-thirds of affected people are men. The five-year survival rate is 75%. It is incurable, but there are many effective treatments.
- ***Acute myelogenous leukemia (AML)*** occurs more commonly in adults than in children, and more commonly in men than women. AML is treated with chemotherapy. The five-year survival rate is 40%.
- ***Chronic myelogenous leukemia (CML)*** occurs mainly in adults. A very small number of children also develop this disease.

Symptoms

All symptoms associated with leukemia can be attributed to other diseases.

- Damage to the bone marrow, by way of displacing the normal bone marrow cells with higher numbers of immature white blood cells, results in a lack of blood platelets, which are important in the blood clotting process. This means people with leukemia may easily become, bleed excessively, or develop pinprick bleeds (petechiae).
- White blood cells, which are involved in fighting pathogens, may be suppressed or dysfunctional. This could cause the patient's immune system to be unable to fight off a simple infection or to start attacking other body cells. Because leukemia prevents the immune system from working normally, some patients experience frequent infection, such as tonsils, or pneumonia.
- Finally, the red blood cell deficiency leads to anemia, which may cause dyspnea and pallor.

Causes and risk factors

There is no single known cause for all of the different types of leukemia.
The different leukemias likely have different causes.
Known causes include:

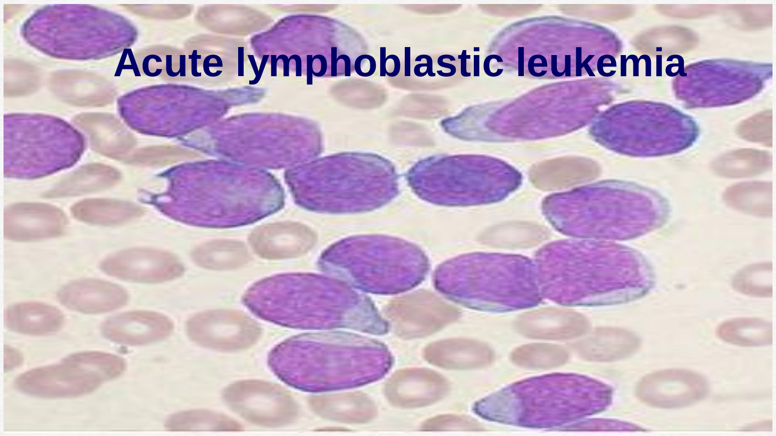
- Natural and artificial ionizing radiation
- Viruses such as Human T-lymphotropic virus
- Some chemicals, notably benzene and alkylating agents 'for previous malignancies
- Genetics; familial incidence, chromosomal abnormalities in leukemic patients.

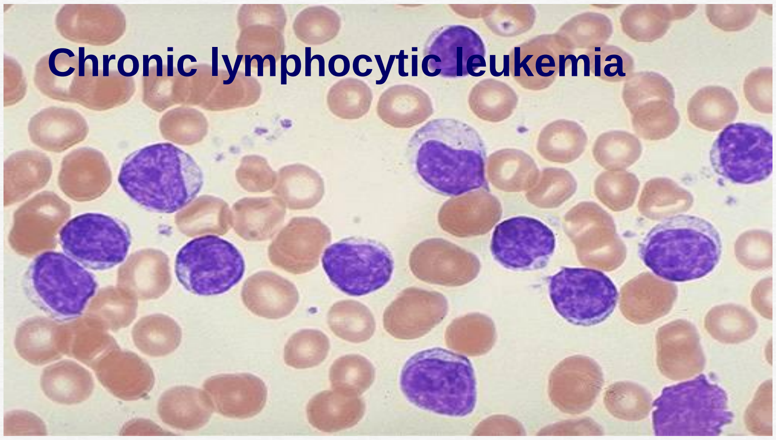
Treatment

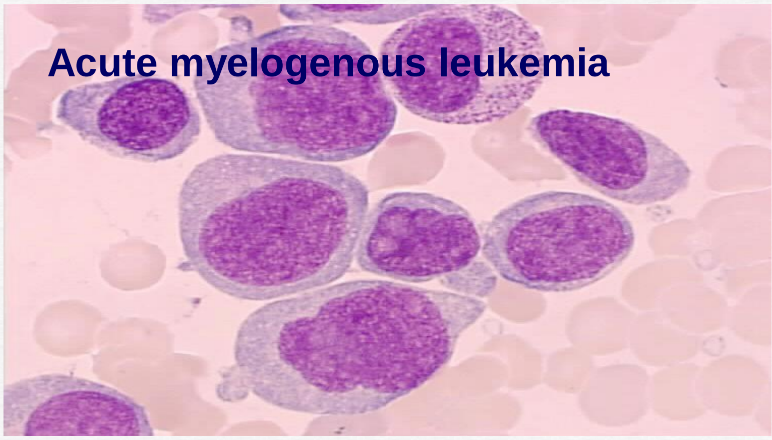
Chemotherapy is the use of drugs to fight cancer. It is the usual treatment for acute leukemia. For most people, that means receiving drugs in three stages:

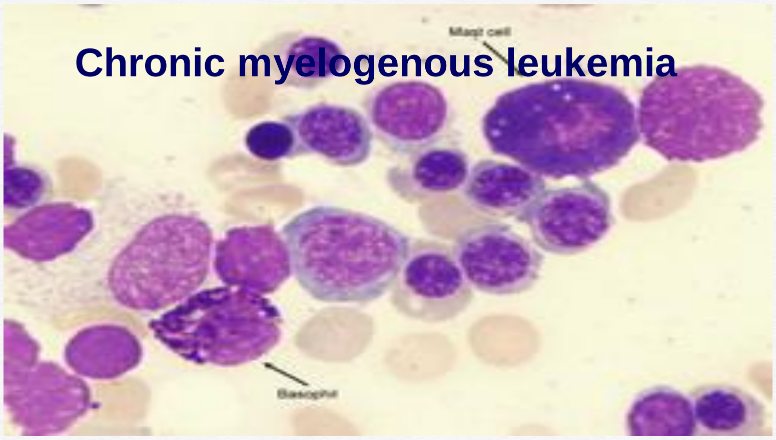
- **Induction.** The goal of this stage is remission. Remission is a period in which the leukemia cells have been destroyed and are replaced with healthy cells. Induction is done with high doses of powerful drugs (such as vincristine, and cyclophosphamide) which may be given over a period of time from about a week to a month, depending on the type of leukemia. Then it takes several weeks for your body to start growing new blood cells.
- **Consolidation.** Even when tests show no leukemia cells can be found, there may still be some left. The goal of consolidation is to kill any cells that are left. Consolidation often involves the same drugs given in the induction stage, but the schedule and doses may be different.
- **Maintenance.** The goal of this stage is to prevent leukemia cells from growing back. During maintenance you are given lower doses of drugs over the course of 2 to 3 years.

Types of leukemia









Platelets abnormalities

Thrombocytosis

Thrombocytosis is the presence of high platelet counts in the blood, and can be either reactive or primary (also termed essential).

Causes

Increase platelet counts can be due to a number of disease processes:

Essential (primary)

- Essential thrombocytosis (a form of myeloproliferative disease)
- Other myeloproliferative disorders such as chronic myelogenous leukemia, and polycythemia vera

Reactive (secondary)

- Inflammation
- Surgery
- Hemorrhage and/or iron deficiency
- Chronic pancreatitis, and inflammatory bowel disease
- Renal failure

Thrompocytopenia

Thrombocytopenia is the presence of relatively few platelets in blood.

Signs and symptoms

Often, low platelet levels do not lead to clinical problems. Occasionally, there may be bruising, particularly purpura in the forearms, nosebleeds and/or bleeding gums.

Causes

Decreased platelet counts can be due to a number of disease processes:

Decreased production

- Decreased production in the bone marrow:
  - Vitamin B12 or folic acid deficiency
  - Leukemia
  - systemic viral or bacterial infection
- Decreased production of thrombopoietin by the liver in liver failure.

Note: *Patients who have a bone marrow disease, such as leukemia or other cancer in the bone marrow, often experience excessive bleeding due to a significantly decreased number of platelets (thrombocytopenia).*

Increased destruction

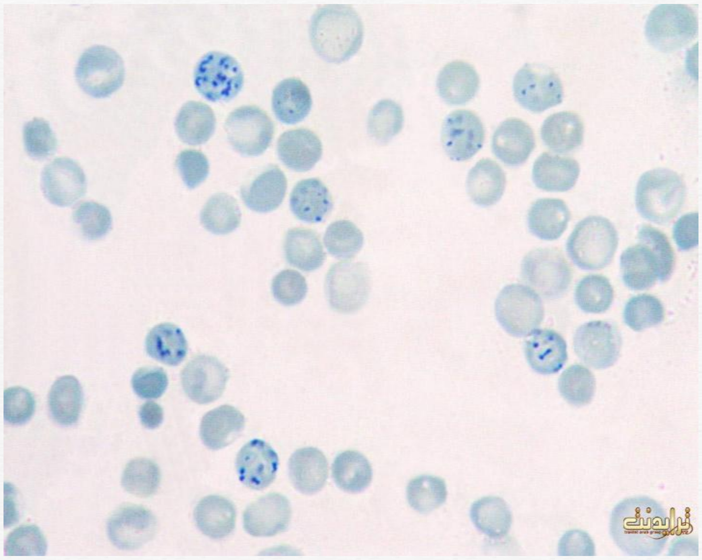
- immune-mediated thrombocytopenic purpura
- Splenic sequestration of platelets due to hypersplenism
- Thrombocytopenia-inducing medications include:
  - Valproic acid
  - Methotrexate
  - Interferon

Note: *If platelet levels fall below 20,000 per microliter, spontaneous bleeding may occur and is considered a life-threatening risk.*

Reticulocyte Count

Overview

Reticulocytes are newly-produced red blood cells. They are slightly larger than totally mature red blood cells, and have some residual ribosomal RNA. The presence of RNA allows a visible blue stain to bind. This allows them to be detected and counted as a distinct population.



Reticulum is present in newly released blood cells for 1 to 2 days before the cell reaches its full mature state.

They are called reticulocytes because of a reticular (mesh-like) network of ribosomal RNA that becomes visible under a microscope with certain stains.

Sample

Use fresh Blood sample collected in EDTA tube (It should be assayed in 6 hours).

Why It Is Done

The reticulocyte count gives an indication about bone marrow function

- The reticulocyte count is used to differentiate anemias caused by bone marrow failure (decreased RBC production) from those caused by hemorrhage or hemolysis (destruction of RBCs),

The increased destruction or loss of red blood cells causes the bone marrow to make more reticulocytes.

- Used to check the effectiveness of treatment in pernicious anemia and folate and iron deficiency (higher reticulocyte count indicates that the treatment is effective),
- Used to assess the recovery of bone marrow function in aplastic anemia
- Used to help monitor bone marrow response and return of normal marrow function following chemotherapy treatment, bone marrow transplant

Procedure

Reagent

Using ***New methylene blue*** or ***brilliant cresyl blue*** stain a supravital stain (i.e. one that stains living material)

Note: *Keep the stain refrigerated and always make filtration before use.*

Staining method

Reticulocytes can be distinguished from mature RBCs because they still contain remnant genetic material (RNA), Circulating reticulocytes lose their RNA becoming mature RBCs.

- Mix equal volume of Blood and Brilliant creysl blue Stain in clean test tube.
- Re-suspend the mixture by gently mixing.
- Incubate the test tube in water bath for 15 min at 37 degree

- Make a blood smear and wait until dray properly.
- Examine under Microscope (100 X).
- Count the number of RBC and Reticulocyte in 10 field.

Reticulocytes are recognized by the violet-blue stained granules of ribosomal RNA (reticulin) they contain.

Note: *Always perform duplicate counts (preferably using a different person for each count). The two counts should agree within 20% of each other.*

Sources of error

- Not mixing adequately the stained blood prior to making the blood films.
- Not counting the cells accurately or counting too few cells.
- Using stain which has become contaminated and has not been filtered before use.
- Confusing precipitated stain for the reticulin of reticulocytes.

Calculations

Calculate the % of Reticulocyte as follow:

Reticulocyte (%) = (Number of Reticulocyte / Number of RBCs) X 100

Normal

- Most RBCs are fully mature before they are released from the bone marrow into the blood, but about 0.5 – 2.5% of the RBCs in circulation will be reticulocytes.
- Newborns have a higher percentage of Reticulocyte (2-6.5%) but the number drops to near adult levels within 2 months.

Note: *Patients who move to higher altitudes may have increased reticulocyte counts as their body adapts to the lower oxygen content of their new location. Smokers also may demonstrate increased number of RBCs and reticulocytes.*

Interpretation

Reticulocyte count is representative of bone marrow condition

RBC production increased, Reticulocyte count increases

RBC production decreases, Reticulocyte count decreases

Disease states with increased Reticulocyte counts (reticulocytosis)

Increased reticulocyte count (***reticulocytosis***) means that increased RBC production is occurring as the bone marrow replaces cells lost or prematurely destroyed, this can occurs in the following conditions:

- After acute hemorrhage
  It should increase within 2-3 days of a major acute hemorrhage, for instance, and reach its peak in 6-10 days.
- Hemolytic Anemias

Reticulocyte responses are higher with haemolysis than haemorrhage.

Note: *Chronic bleeding (hemorrhage) or hemolysis (RBC destruction). The body compensates for this loss by increasing the rate of RBC production. When this happens, the number and percentage of reticulocytes in the blood increases until the balance is restored or until the production capacity of the marrow is reached.*

- After adequate doses of iron in iron-deficiency anemia, the rise in reticulocytes may exceed 20%.
- There is a proportional increase when pernicious anemia is treated by transfusion or vitamin B12 therapy.

Disease states with decreased Reticulocyte counts (Reticulocytopenia)

Decreased reticulocyte counts (***Reticulocytopenia***) may occur when the bone marrow is not functioning normally due to a bone marrow disorder such as:

- Aplastic Anemia (a persistent deficiency of reticulocytes suggests a poor prognosis)
- Pernicious Anemia or iron deficiency anemia before treatment
- Attributed to chemotherapy or radiation treatments for cancer
- Problems of erythropoietin production

Reticulocyte Index

The Reticulocyte production index (RPI, also called a corrected reticulocyte count) is a calculated value used in the diagnosis of anemia. This calculation is necessary because the raw reticulocyte count is misleading in anemic patients. The problem arises because the reticulocyte count is not really a count but rather a percentage.

In anemia, the patient's red blood cells are depleted, creating an erroneously elevated reticulocyte count.

The RI is a measurement that corrects the reticulocyte count for anemia, giving a more accurate picture of reticulocyte count.

Reticulocyte Production Index is calculated as follows:

Reticulocyte index = Reticulocyte count X (Patient HCT / Normal HCT)

The next step is to correct for the longer life span of prematurely released reticulocytes in the blood--a phenomenon of increased red blood cell production. This relies on a table:

Hematocrit (%)	Retic survival(days) = maturation correction
36-45	1.0
26-35	1.5
16-25	2.0
15 and below	2.5

RPI = Reticulocyte index / Maturation factor

Normal

The reticulocyte index (RI) should be between 1.0 and 2.0 for a healthy individual.

Interpretation of RPI

RI < 1 with anemia indicates decreased production of reticulocytes and therefore red blood cells. (hypoproliferative component to anemia)

RI > 2 with anemia indicates loss of red blood cells (destruction, bleeding, etc) leading to increased compensatory production of reticulocytes to replace the lost red blood cells.

Coagulation profile

The ability of the body to control the flow of blood following vascular injury is paramount to continued survival. The process of blood clotting and then the subsequent dissolution of the clot, following repair of the injured tissue, is termed hemostasis.

Hemostasis Mechanism

Hemostasis is a complex process which causes the bleeding process to stop. Hemostasis, composed of 4 major events that occur in a set order following the loss of vascular integrity:

Vascular spasm (Vessel constriction)

- Cutting or damaging blood vessels leads to vascular spasm of the smooth muscle in the vessel wall.
- This produces a vasoconstriction which will slow or even stop blood flow. This response will last up to 30 minutes and is localized to the damaged area.
- *vascular constriction is due to:*
  - nervous sympathetic reflex which initiated by pain impulse
  - local myogenic contraction of vascular wall due to trauma
  - serotnine and prostaglandins released by platelets

Platelet plug formation (primary hemostasis)

- Damaged endothelial cells lining the blood vessel release von Willebrand's Factor.
- This substance makes the surfaces of the endothelial cells "sticky".
- Within seconds of cutting the blood vessel, the platelets stick to the endothelial surface and clump together around the cut.
- The platelets that adhere to the vessel walls now begin to secrete Adenosine diphosphate (ADP) and thromoboxan A2 which causes the aggregation of nearby free platelets which attach to the fixed platelets to form platelet plug (*primary hemostasis*).

Blood coagulation and fibrin generation

- It Begins 30 seconds to several minutes after phases I and II have commenced.
- The essential reaction in coagulation is the conversion of fibrinogen to fibrin by thrombin enzyme which is formed from the inactive prothrombin
- Activation of prothrombin is depends on the presence of several clotting factors derived from damaged tissue , platlets , and plasma itself.

There are two pathways leading to the formation of the thrombus:

- ***Extrinsic Pathway***
- the tissue factor pathway (also known as the extrinsic pathway).
- Following damage to the blood vessel, Proconvertin (VII) leaves the circulation and comes into contact with tissue factor (TF), forming an activated complex (TF-FVIIa).
- TF-FVIIa activates Antihemophilic factor B (IX) and Stuart-Prower factor (X).
- FXa and its co-factor Proaccelerin (V) form the prothrombinase complex, which activates prothrombin to thrombin.

> **Note:** *The extrinsic pathway is a very rapid process, i.e., within 12 to 15 seconds. However, the production of Thrombin is low and the resulting clot is small. This pathway is most effective as a "quick patch" process and It is now known that the primary pathway for the initiation of blood coagulation is the tissue factor pathway.*

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Intrinsic Pathway

- These are the contact activation pathway (also known as the intrinsic pathway)
- The formation of a red thrombus or a clot in response to an abnormal vessel wall in the absence of tissue injury is the result of the intrinsic pathway.
- The contact activation pathway Is initiated by the blood coming in contact with exposed collagen in the blood vessel wall with formation of the primary tenase complex as follow:
- Prekallikrein is converted to kallikrein and Hageman factor (XII) becomes active.
- Active factor XII converts Plasma thromboplastin antecedent (XI) into its active form.
- XI activates Antihemophilic factor B (IX), which with its co-factor Antihemophilic factor A form the tenase complex, which activates Stuart-Prower factor (X) to its active form.

> **Note:** This process is considerably slower (5 to 10 minutes) but results in the formation of larger amounts of thrombin. This allows the formation of larger clots.

> **Note:** The intrinsic pathway has low significance under normal physiological conditions. Most significant clinically is the activation of the intrinsic pathway by contact of the vessel wall with lipoprotein particles, VLDLs and chylomicrons. This process clearly demonstrates the role of hyperlipidemia in the generation of atherosclerosis.

Common Pathway

Factor X (active) initiates the conversion of Prothrombin to active form of the enzyme Thrombin.
Thrombin accelerates the formation of thin fibrin threads from soluble protein fibrinogen
The strands of fibrin form a meshwork, and trap blood cells and platelets which form into a solid clot. (Secondary hemostasis)

Clot dissolutionFibrinolysis

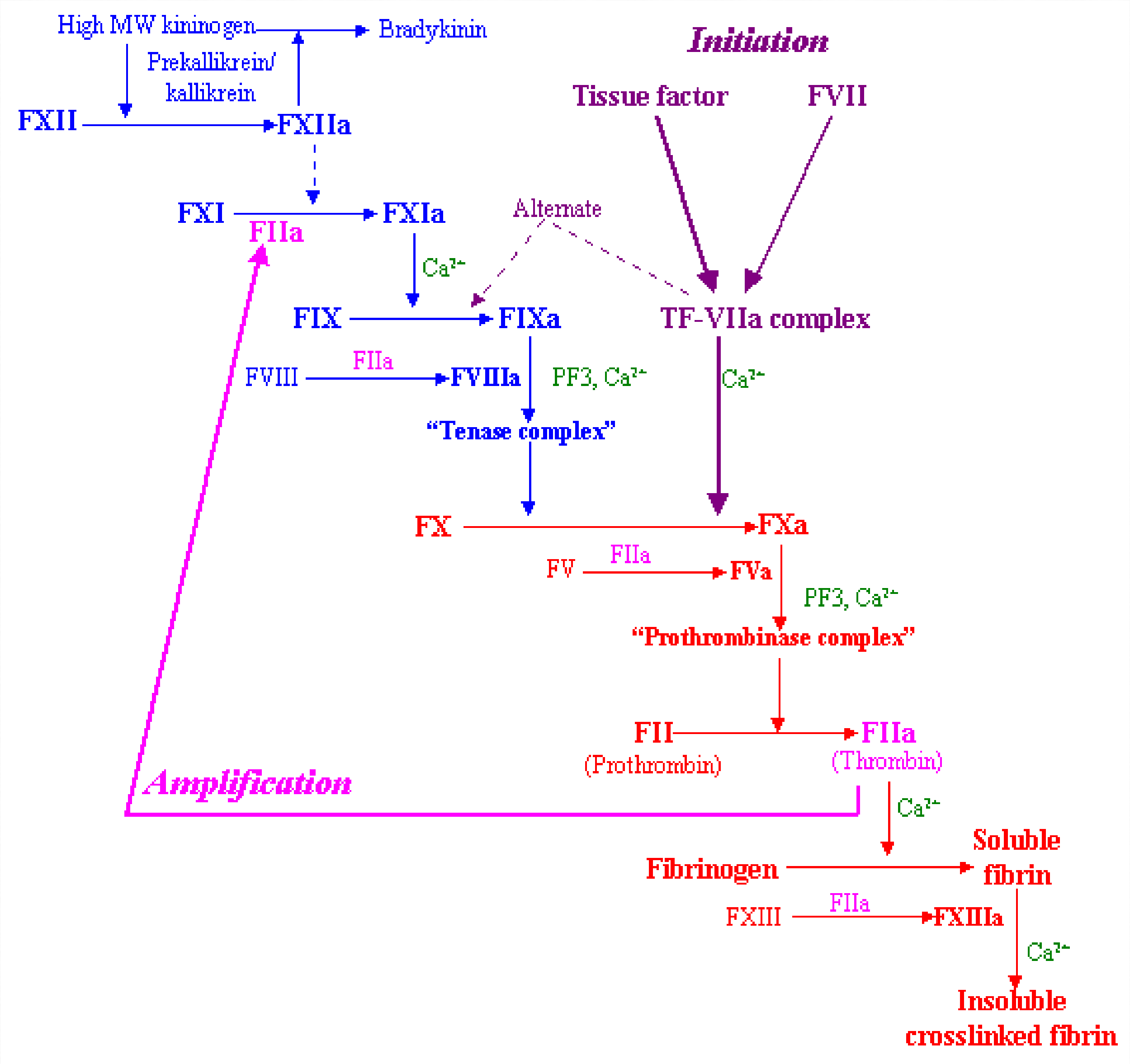
- Finally, the clot must be dissolved in order for normal blood flow to resume following tissue repair. The dissolution of the clot occurs through the action of plasmin
- plasmin is a serine protease that circulates as the inactive proenzyme, plasminogen.
- Plasminogen binds to both fibrinogen and fibrin, thereby being incorporated into a clot as it is formed.
- Tissue plasminogen activator (tPA) convert plasminogen to plasmin.
- plasmin then digests the fibrin; the result is soluble degradation product to which neither plasmin nor plasminogen can bind.
- Following the release of plasminogen and plasmin they are rapidly inactivated by their inhibitors.

Why coagulation profile is done?

- Determine a possible cause of abnormal bleeding or bruising.
- Screen for deficiencies of certain blood clotting factors. The lack of some clotting factors can cause bleeding disorders such as hemophilia.
- Screen for conditions that cause abnormal clotting or blood clot formation. Conditions such as antiphospholipid antibody syndrome or lupus anticoagulant syndrome develop when the immune system produces antibodies that attack certain blood clotting factors, causing the blood to clot easily in veins and arteries.
- Screen blood clotting time before a surgery.

Coagulation factors

Factor	Common Name
I	Fibrinogen
II	Prothrombin
III	Tissue factor
IV	Ca2+
V	Proaccelerin
VII	Proconvertin
VIII	Antihemophilic factor A,
IX	Antihemophilic factor B, Christmas factor
X	Stuart-Prower factor
XI	Plasma thromboplastin antecedent (PTA)
XII	Hageman factor
XIII	Fibrin stabilizing factor (FSF)



Bleeding time (CT)

Overview:

Bleeding time is a screening test performed by measuring how long it taken for bleeding to stop from a fresh cut of determined size

The test investigate the efficiency of haemostatic mechanisms other than blood clotting especially vascular spasm and formation of platelets plug

Why Get Tested?

- This test is useful for detecting bleeding disorders.

  Note: *in the cases of bleeding disorders the patients may present with unexplained nosebleeds (epistaxis), excessive or prolonged menstrual blood flow (menorrhagia), or prolonged bleeding after minor cuts, tooth brushing or trauma. Other patients may have unexplained skin lesions, including petechiae (small intradermal or mucosal hemorrhages), purpura (areas of mucosal or skin hemorrhage larger than petechiae)*

- Before surgery, in certain circumstances, to assess your risk of bleeding problems during an operation.

Sample:

The test is done by making Finger stick or Finger prick

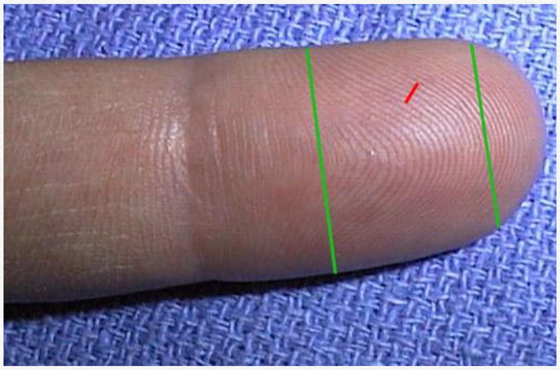
Procedure:

Duke Method

With the Duke method, the patient is pricked with a special needle or lancet, preferably on the earlobe or fingertip, after having been swabbed with alcohol. The prick is about 3-4 mm deep. The patient then wipes the blood every 30 seconds with a filter paper. The test ceases when bleeding ceases.

Procedure

1. Prepare equipments of the test as sterile lancet, slides, cotton, stop watch, and so on.
2. Verify the patient's condition.
3. Position the patient.
4. Chose the site
   - *Do not use the tip of the finger or the center of the finger (it is more sensitive and cause much pain).*

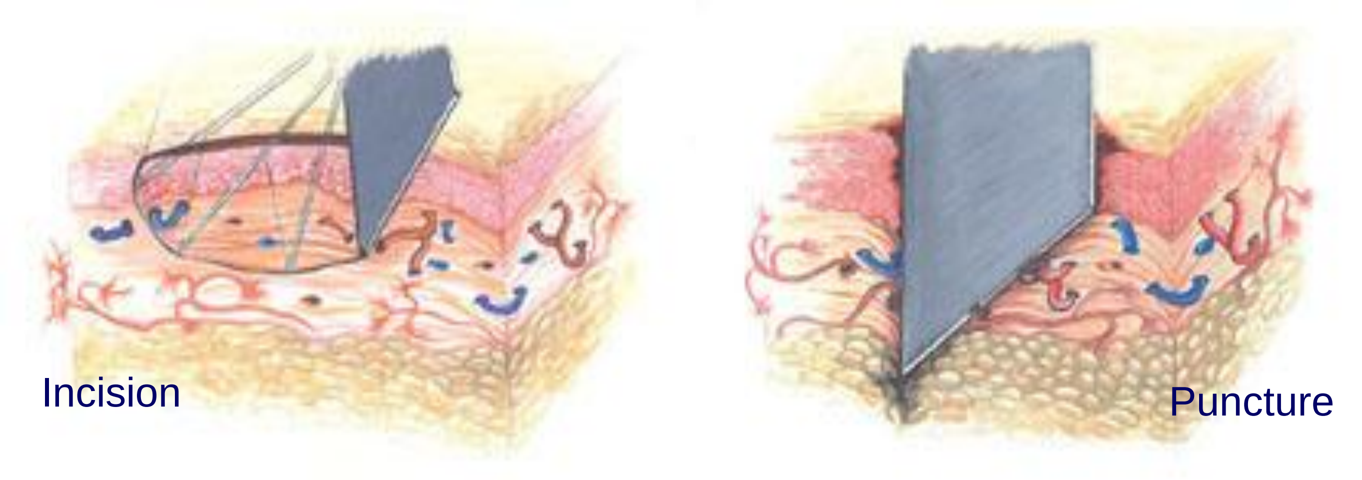


5. Clean the finger using ethanol.
6. Using a sterile lancet, make a quick skin puncture
   - Puncture should be 2mm deep (it should be deep enough so that blood flow freely with out squeezing the stabbed site)
   - It should not exceed 2.5 mm to avoid bone

7. Gently pressure will aid if blood do not flow freely
8. Do not squeeze the finger

   Note: *Squeezing lead to sample dilution with interstitial fluid and also help blood clotting*

9. Wipe away the drop of blood every 30 seconds with a filter paper or piece of cotton.



Normal:

The normal time is about 2-5 minutes.

Note: *it should also be noted that the depth of the puncture or incision may be the source of error.*

Interpretation:

A prolonged bleeding time may be a result from:

- Decreased number of thrombocytes (thrombocytopenia)
- Von Willebrand disease, a relatively common inherited bleeding disorder (von Willebrand factor is responsible for helping platelets stick to the injured blood vessel wall and to each other)
- Hypofibrinogenemia
- haemophilia
- Cyclooxygenase inhibitors as Aspirin can prolong bleeding time significantly.

Note: *Oral anticoagulant therapy and heparin have their major effects on coagulation factors and do not prolong bleeding time*

Disadvantage:

- There is a poor correlation between the degree of prolongation of the bleeding time and the degree of clinical bleeding
- Bleeding time is insensitive to platelet dysfunction as many patients with congenital and acquired platelets dysfunction will have normal bleeding time

Clotting time (CT)

Overview:
It is the whole blood clotting time which investigate the efficiency of blood coagulation

Why Get Tested?
- If you have certain liver diseases that can affect the making of blood clotting factors.
- Before surgery, in certain circumstances, to assess your risk of bleeding problems during an operation
- If you take anticoagulant medication (to check that you are taking the correct dose)

Procedure:
Lee-white method
- Blood sample is collected from the vein (about 0.5 ml)
- The blood is placed into a glass tube
- The tube is warmed to 37 degree
- Tilt the tube every 30 seconds interval until blood clots

Sources of error
- Low blood volume will give short time
- Low incubation temperature will shorten clotting time
- Unnecessary agitation of blood sample shorten clotting time

Normal:
The normal time is about 5-10 minutes.

Interpretation:
- Genetic conditions where one or more of the clotting factors is missing. The most well known is haemophilia A which occurs in people who do not make factor VIII.
- Lack of vitamin K can cause bleeding problems as you need this vitamin to make certain clotting factors.
- Liver disorders can sometimes cause bleeding problems as your liver makes most of the clotting factors.
- Anticoagulant medication can affect the blood clotting

Disadvantage:
- It is non specific test and do not till which factor is missing
- Only severe clotting factors defects is recognized by this method

Prothrombin time (PT)

Overview:

The prothrombin time (PT) and its derived measures of international normalized ratio (INR) are measures of the extrinsic pathway of coagulation. They are used to determine the clotting tendency of blood, in the measure of anticoagulant dosage, liver damage, and vitamin K status.

PT measures factors I, II, V, VII, and X.

Sample:

The prothrombin time is most commonly measured using blood plasma. Blood is drawn into a test tube containing liquid citrate, which acts as an anticoagulant by binding the calcium in a sample.

The blood is mixed, then centrifuged for about 10 minutes then separate blood cells from plasma as soon as possible.

Excess citrate or insufficient blood can cause incorrect result

Procedure:

- **Reagent :** Thromboplastin with calcium prepared from rabbit brain
- **Principle:**

An excess of calcium is added (thereby reversing the effects of citrate), which enables the blood to clot again. Tissue factor (also known as factor III) is added, and the time the sample takes to clot is measured.

- **Manual test:**
  - Incubate 200 μl reagent at 37 degree for 15 min
  - Incubate 100 μl plasma at 37 degree for 15 min
  - Add the plasma to the reagent and start the stop watch simultaneously and note the clotting time

Perform each test in duplicate

- **Automated coagulation machines:**

Principle

Automated coagulation machines or Coagulometers measure the ability of blood to clot (these equipments measure the polymerization of fibrin monomers).

Depending on the test, different substances can be added to the blood plasma to trigger a clotting reaction.

The progress of clotting may be monitored by two methods:

Mechanical monitoring

This method use a vibrating probe which is suspended in a reaction cup. The movement of the probe is continuously measured through a magnetic system.

As the fibrin monomer polymerization proceeds and viscosity increase , there is increased resistance to the movement of the probe until it stop. This impedance to the movement of the probe is recorded

Optical monitoring

The characteristic of fibrin monomer polymerization can be determined by measuring the changing turbidity of the plasma due to the formation of fibrin polymer

The growing polymer will take a light scattering characteristics and this scattered light as well as transmitted light can be recorded at 650 nm by photo detectors

Results

Expressed as a percentage of activity compared to the control

International normalised ratio (INR)

The result (in seconds) for a prothrombin time performed on a normal individual will vary according to the type of analytical system employed. This is due to the variations between different batches of manufacturer's tissue factor used in the reagent to perform the test. The INR was devised to standardize the results. Each manufacturer assigns an ISI value (International Sensitivity Index) for any tissue factor they manufacture. The ISI value indicates how a particular batch of tissue factor compares to an internationally standardized sample. The ISI is usually between 1.0 and 2.0. The INR is the ratio of a patient's prothrombin time to a normal (control) sample, raised to the power of the ISI value for the analytical system used.

The INR uses the ISI to equate all thromboplastins to the reference thromboplastin through the following equation:

$$\mathrm{INR} = \left(\frac{\mathrm{PT_{test}}}{\mathrm{PT_{normal}}}\right)^{\mathrm{ISI}}$$

Normal:

The reference range for prothrombin time is usually around 11–16 seconds (70% - 100%)
The normal range for the INR is 0.8–1.2

INR for those receiving therapeutic anticoagulation therapy is 2.0 to 3.0. For those with mechanical prosthetic heart valves, an INR of 2.5 to 3.5 is suggested

Interpretation:

The prothrombin time is the time it takes plasma to clot after addition of tissue factor (obtained from animals). This measures the quality of the extrinsic pathway (as well as the common pathway) of coagulation. The speed of the extrinsic pathway is greatly affected by levels of factor VII in the body. Factor VII has a short half-life and its synthesis requires vitamin K.

The prothrombin time can be prolonged in the following conditions:

- anti-coagulant therapy
- as a result of deficiencies in vitamin K, which can be caused by malabsorption, or lack of intestinal colonization by bacteria (such as in newborns)
  (the production of factors II, VII, IX, and X requires Vitamin K).
- poor factor VII synthesis (due to liver disease as hepatitis and cirrhosis)
- increased factor VII consumption (in disseminated intravascular coagulation)
- Deficiency of factors X , II , V , I

Disseminated Intravascular Coagulation (DIC)

DIC is an acquired hemorrhagic syndrome characterized by uncontrolled formation and deposition of fibrin thrombi. Continuous generation of thrombin causes depletion (consumption) of the coagulation factors and results in uncontrolled bleeding. Also, fibrinolysis is activated in DIC. This further adds to the hemostasis defect caused by the consumption of clotting factors. The many coagulation test abnormalities found in acute DIC include the following:

Prolonged PT , PTT , BT
Decreased Fibrinogen , Platelet count and clotting factors II, V, VIII, and X
Positive Fibrin degradation products and D-Dimer

Partial thromboplastin time (PTT)

Overview:

The partial thromboplastin time (PTT) or activated partial thromboplastin time (aPTT or APTT) is a performance indicator measuring the efficacy of both the "intrinsic" (now referred to as the contact activation pathway) and the common coagulation pathways.

The test is termed "partial" due to the absence of tissue factor from the reaction mixture.

Why Get Tested?

- When a patient is on intravenous (IV) or injection heparin therapy, the aPTT is ordered at regular intervals to monitor the degree of anticoagulation.

  Note: *When a person is switched from heparin therapy to longer-term anticoagulant therapy, both the aPTT and PT are monitored until the patient has stabilized.*

- It is often used with recurrent miscarriages that often are associated with anticardiolipin and phospholipid antibodies.
- The aPTT and PT tests are also sometimes used as pre-surgical screens for bleeding tendencies.

Procedure:

In order to activate the intrinsic pathway, phospholipid, an activator (such as silica) which activate the contact depending factor XII, and calcium (as calcium chloride to reverse the anticoagulant effect of the oxalate) are mixed into the plasma sample . The time is measured until a thrombus (clot) forms. This testing is performed by a medical technologist.

Normal:

Partial thromboplastin time (PTT): 30 - 45 seconds

Interpretation:

Prolonged APTT may indicate:

- Use of heparin anticoagulant therapy

The heparin dosage for people being treated to prevent the formation of blood clots is usually adjusted so that the PTT is about 1.5 to 2.5 times the normal value.

- Disseminated intravascular coagulation (DIC) that may cause both bleeding and clotting as factors are used up at a rapid rate
- Deficiencies of clotting factors VIII, IX, XI and XII
- Prolonged in cases of Haemophilia and rarely von Willebrand factor.
- In cases of antiphospholipid antibody syndrome and lupus anticoagulant syndrome that can cause abnormal clotting. These syndromes are a complication of lupus in which the immune system produces antibodies that attack certain blood clotting factors, causing the blood to clot easily in veins and arteries and may leads to recurrent miscarriages
- Chronic condition such as liver disease ,disease (such as nephrotic syndrome),

  Note: *PTT is not affected in early Liver failure (put PT prolonged) and both PT , PTT are prolonged in end-stage Liver failure*

  Note: *If the aPTT is abnormal and the PT is normal, you may have deficiencies of factors VIII, IX, XI, or XII.*
  If the aPTT is normal and the PT is prolonged, you may have a deficiency of factors I, II, V, VII, or X.
  If both PT and aPTT are abnormal, you may have deficiencies in the common pathway or to multiple factor deficiencies.

Blood group

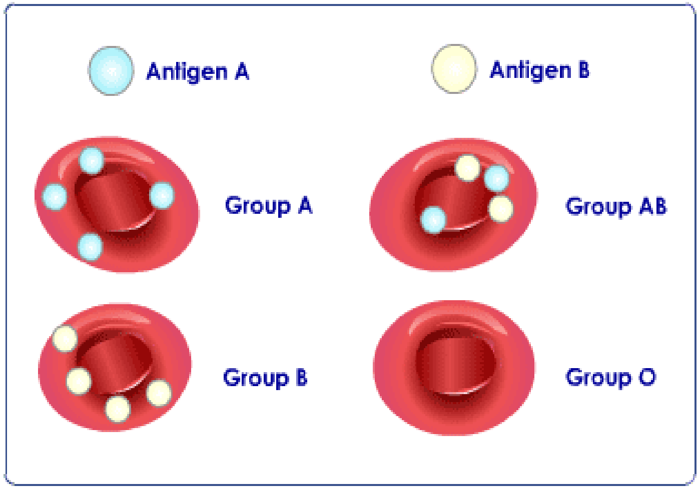
Overview

What is a blood group?

Red blood cells contain certain proteins on their surface. These proteins are called antigens. There are various types of red blood cell antigens - the ABO and rhesus types are the most important.

ABO antigens

The two major antigens or surface identifiers on human RBCs are the A and B antigens. Blood is grouped according to the presence or absence of these antigens.



ABO antibodies (Isohaemagglutinins)

Anti-A and Anti-B, the common IgM antibodies to the RBC surface antigens of the ABO blood group system, are sometimes described as being "naturally occurring"; however, this is a misnomer, because these antibodies are formed in infancy by sensitization in the same way as other antibodies.

The theory that explains how these antibodies are developed states that antigens similar to the A and B antigens occur in nature, including in food, plants, and bacteria. After birth (during the first years of life) an infant's gut becomes colonized with normal flora that express these A-like and B-like antigens, causing the immune system to make antibodies to those antigens that the red blood cells do not possess. People who are blood type A will have Anti-B antibodies, blood type B will have Anti-A antibodies, blood type O will have both Anti-A and Anti-B antibodies, and blood type AB will have neither.

These antibodies are of the IgM class, which have the capability of agglutinating (clumping) and damaging red blood cells within the blood vessels, possibly leading to death.

The ABO system

According to the AB0 blood group system there are four different kinds of blood groups: A, B, AB or 0

- ***A blood group individuals*** will have type A antigens on the surface of red blood cells and anti-B antibodies in plasma.
- ***B blood group individuals*** will have type B antigens on the surface of red blood cells and anti-A antibodies in your plasma.
- ***AB blood group*** are those who will have type A and type B antigens on the surface of red blood cells and no antibodies to A or B antigens in plasma.
- ***O blood group*** are those who will have neither type A or type B antigens on the surface of red blood cells but will have anti-A and anti-B antibodies in plasma.

Blood Group	Antigens on RBCs	Antibodies in Serum	Genotypes
A	A	Anti-B	AA or AO
B	B	Anti-A	BB or BO
AB	A and B	Neither	AB
O	Neither	Anti-A and Anti-B	OO

When to Get Tested?

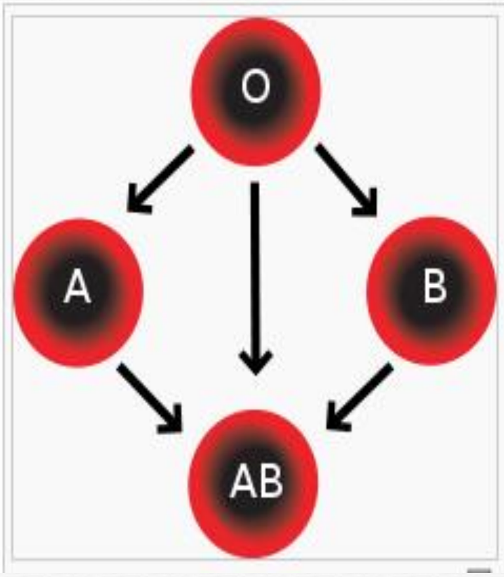
➤ When need to be transfused with blood or blood components

Cases in which blood or blood products are needed for treatment may include:

- Severe anemia
- Surgical patients with intra-operative or post-operative bleeding
- Injury or trauma patients with excessive blood loss
- Bleeding disorders such as hemophilia

Who can receive blood from whom?

Of course you can always give A blood to persons with blood group A, B blood to a person with blood group B and so on. But in some cases you can receive blood with another type of blood group, or donate blood to a person with another kind of blood group.



Universal blood donor

A universal blood donor is someone who has an O blood type and is Rh negative. This means that they have no A or B antigens or Rh factor on their red blood cells. Their red blood cells can be given to a patient with any ABO or Rh blood type

Universal blood recipient

A universal recipient is a person who has an AB blood type and is Rh positive. They can receive red blood cells of any ABO or Rh type with no risk

Recipient [1]	Donor[1]							
	O−	O+	A−	A+	B−	B+	AB−	AB+
O−	✓							
O+	✓	✓						
A−	✓		✓					
A+	✓	✓	✓	✓				
B−	✓				✓			
B+	✓	✓			✓	✓		
AB−	✓		✓		✓		✓	
AB+	✓	✓	✓	✓	✓	✓	✓	✓

Inheritance of Blood groups

ABO blood types are controlled by a single gene (the ABO gene) with three alleles(A, B, or O) on ***chromosome 9***, and they do not change as a result of environmental influences during life.

- ***Both A and B alleles are dominant over O.*** As a result, individuals who have an AO genotype will have an A phenotype. People who are type O have OO genotypes. In other words, they inherited a recessive O allele from both parents.
- ***The A and B alleles are codominant.*** Therefore, if an A is inherited from one parent and a B from the other, the phenotype will be AB.

The possible outcomes are shown below:

Blood Type		Mother's Type			
		O	**A**	**B**	**AB**
Fathers' Type	O	O	O, A	O, B	A, B
	A	O, A	O, A	O, A, B, AB	A, B, AB
	B	O, B	O, A, B, AB	O, B	A, B, AB
	AB	A, B	A, B, AB	A, B, AB	A, B, AB

What happens when blood clumps or agglutinates?

For a blood transfusion to be successful, ABO and Rh blood groups must be compatible between the donor blood and the patient blood. If they are not, the red blood cells from the donated blood will clump or agglutinate.

The A antigen and the A antibodies can bind to each other in the same way that the B antigens can bind to the B antibodies. The red blood cells will be linked together, , by the antibodies.

The agglutinated red cells can clog blood vessels and stop the circulation of the blood to various parts of the body. The agglutinated red blood cells also crack and its contents leak out in the body. The red blood cells contain hemoglobin which becomes toxic when outside the cell. This can have fatal consequences for the patient.

Sample

Whole blood (EDTA anticoagulant)

Procedure

Reagents for ABO Typing

The monoclonal Anti-A, Anti-B reagents are murine monoclonal antibodies of IgM class, secreted by mouse hybridoma cell lines, which were selected for their ability to agglutinate specifically human RBCs bearing blood group antigens A and B on direct agglutination tests (slide, microplate and tube test).

Assay method

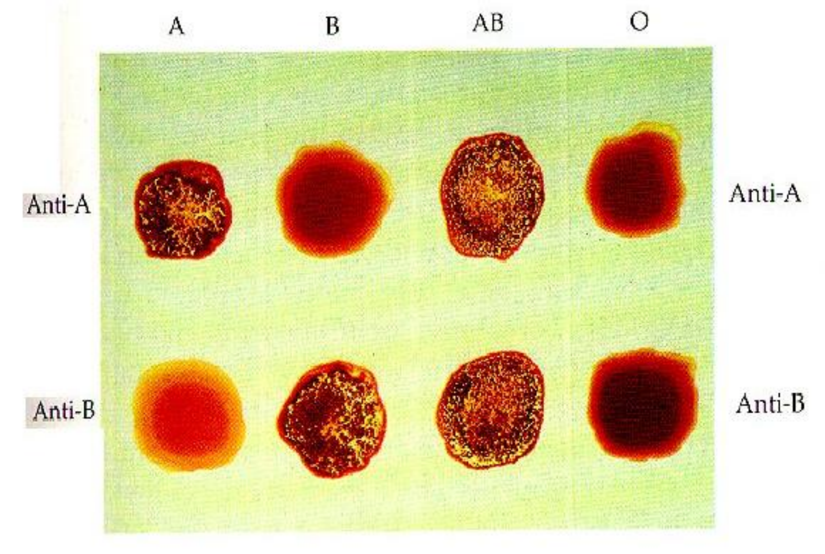
- On a clean slide, carefully put two drops of blood and make sure that the drops do not touch one another.
- Add a drop of anti-A to the first blood drop ,mix the two drops,

using a wooden stick. Check and see if agglutination occurred.

- Add a drop of anti-B to the second blood drop ,mix the two drops, using a wooden stick. Check and see if agglutination occurred.

Results

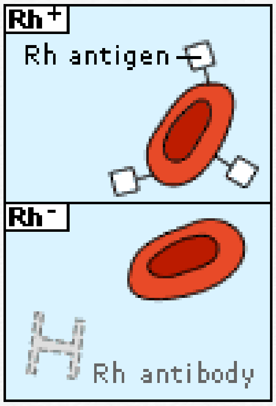
Agglutination with Anti-A	Agglutination with Anti-B	Blood type
+	-	A
-	+	B
+	+	AB
-	-	O



Rhesus factor

Overview

The Rhesus system is named after the ***Rhesus monkey***, following experiments by Karl Landsteiner and Alexander S. Wiener, which showed that rabbits, when immunised with rhesus monkey red cells, produce an antibody that also agglutinates the red blood cells of many humans.



Many people have a so called Rh factor on the red blood cell's surface. This is also an antigen and those who have it are called Rh+. Those who haven't are called Rh-.

Rh antigens

The proteins which carry the Rhesus antigens are transmembrane proteins. The main antigens are C, D, E, c and e, which are encoded by two gene loci, the *D* locus and the *CE* locus.

Note: *There is no d antigen. Lowercase "d" indicates the absence of the D antigen (the gene is either deleted or nonfunctional).*

Note: *The terms Rhesus factor and Rh factor are equivalent and refer to the Rh D antigen only.*

Rhesus genotypes		
Genotype	**symbol**	**Rh(D) status**
cde/cde	rr	Negative
CDe/cde	R_1r	Positive
CDe/CDe	R_1R_1	Positive
cDE/cde	R_2r	Positive
CDe/cDE	R_1R_2	Positive
cDE/cDE	R_2R_2	Positive

Rh antibodies

Unlike the ABO antigens, the only ways antibodies are developed against the Rh factor are through placental sensitization or transfusion.

These antibodies is of IgG type

Note: *Rh-positive blood is more common than Rh-negative blood. About 85% of people are Rh positive.*

Inheritance of Rhesus factor

The Rh(D) antigen is inherited as one gene (RHD) (on the short arm of the first chromosome) with two alleles, of which Rh+ is dominant and Rh- recessive.
The gene codes for the RhD polypeptide on the red cell membrane. Rh- individuals who lack a functional RHD gene (dd genotype) do not produce the D antigen, and may be sensitized to Rh+ blood.

Rh Factor		**Mother's Type**	
		Rh +	**Rh -**
Father's Type	**Rh +**	Rh +, Rh -	Rh +, Rh -
	Rh -	Rh +, Rh -	Rh -

Who can receive blood from whom?

- A person with Rh+ blood can receive blood from a person with Rh- blood without any problems.
- A person with Rh- blood does not have Rh antibodies naturally in the blood plasma (as one can have A or B antibodies, for instance). But a person with Rh- blood can develop Rh antibodies in the blood plasma if he or she receives blood from a person with Rh+ blood, whose Rh antigens can trigger the production of Rh antibodies.

When to Get Tested?

- Before a person receives a blood transfusion.
- Before surgery.
- When a woman is planning to become pregnant or first becomes pregnant.

What Affects the Test

Factors that can interfere with your test and the accuracy of the results include:

- Taking some antibiotics, such as cephalexin, which may lead to a false Rh-positive result
- Receiving a blood transfusion within the 3 months before a blood type test.
- Having a bone marrow transplant.

Erythroblastosis fetalis

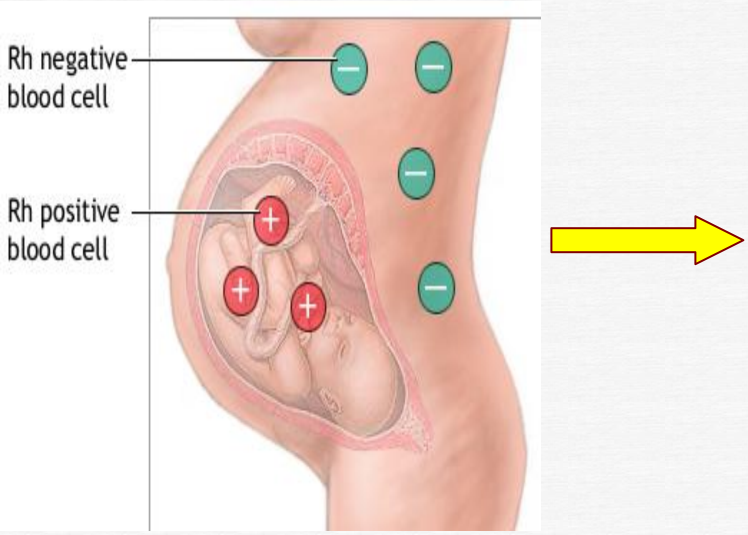
This condition occurs when there is an incompatibility between the blood types of the mother and the baby. The disorder in the fetus due to rhesus-D incompatibility is known as hemolytic disease of the newborn or ***erythroblastosis fetalis.***

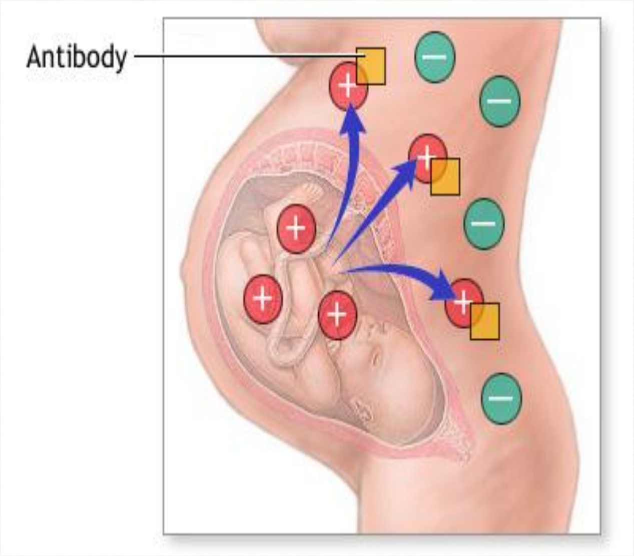
erythroblastosis refers to the making of immature red blood cells, fetalis refers to the fetus

Pathology

If the woman is Rh positive, or if both she and the baby's father are Rh negative, there is no reason to worry about Rh incompatibility.

Rh incompatibility occurs when the mother's blood type is Rh negative and her fetus's blood type is Rh positive.

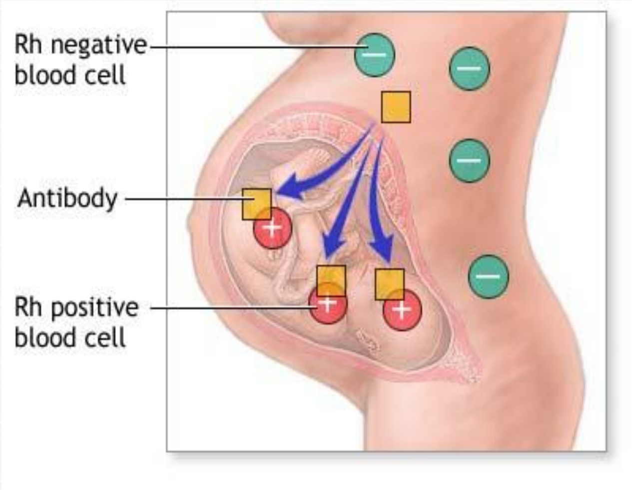




If the blood of an Rh-positive baby mixes with the blood of an Rh-negative woman during pregnancy or delivery, the mother's immune system produces antibodies. This antibody response is called Rh sensitization leading to the production of maternal IgG anti-RhD antibodies which can pass through the placenta.

Note: *Your first baby is usually safe, because fetal and maternal blood usually do not mix until delivery.*

If your second baby is also Rh positive, there's a risk that your antibodies will attack her blood cells and cause problems.



Symptoms

Symptoms and signs in the Fetus:

- Enlarged liver, spleen, or heart and fluid buildup in the fetus' abdomen seen via ultrasound.

Symptoms and signs in the Newborn:

- Anemia which creates the newborn's pallor (pale appearance)
- Jaundice or yellow discoloration of the newborn's skin, sclera or mucous membrane
- Enlargement of the newborn's liver and spleen
- The newborn may have severe edema of the entire body.
- Dyspnea or difficulty breathing.

Treatment

After birth, treatment depends on the severity of the condition, but could include, phototherapy, transfusion with compatible packed red blood, exchange transfusion with a blood type compatible with both the infant and the mother

Prevention (Rh sensitization)

Rho(D) Immune Globulin is a medicine given by intramuscular injection which is used to prevent the hemolytic disease of newborn.

Mechanism

Rhesus-negative mothers who have had a pregnancy with a rhesus-positive infant are given Rh immune globulin (RhIG) within 72 hours after delivery to prevent sensitization to the D antigen. It works by binding any fetal red cells with the D antigen before the mother is able to produce an immune response and form anti-D IgG.

If it is not given before 72 hours, every effort should still be made to administer the anti-D Ig, as a dose given within 9-10 days may provide some protection (Grade B recommendation).

The treatment , of course, has to be repeated at the end of each pregnancy.

Anti-D Ig should also be given to rhesus-negative women after abortion.

Coombs test

The Coombs' test is an Anti human Globulin test used to detect the presence of anti-bodies that coat and damage red blood cells . This test can detect Rh antibodies in maternal blood, and can be used to diagnose and screen for autoimmune hemolytic anemia

There are two types of Coombs' tests :

❖ The indirect Coombs' test

It is used to identify circulating or serum antibodies against red blood cells. Circulating antibodies against red blood cells may occur in Rh-negative women who carry Rh-positive babies. In this situation, the women may develop antibodies to Rh-positive blood groups. The indirect Coombs' test may be used to identify the presence of anti-D in the mother's blood.

❖ The direct Coombs' test

The direct Coombs' test is performed when the patient's red blood cells are suspected of being covered with auto-antibodies against red blood cells.

This test is applied to the cord blood of babies suspected of suffering from ***erythroblastosis***.

The direct antiglobulin test is used primarily to help determine the cause of hemolytic anemia, in which red blood cells (RBCs) are being destroyed more quickly than they can be replaced (***Immune-related hemolytic anemia***).

Normal results

The absence of agglutination for both the direct and indirect Coombs' test is a normal result and is reported as negative.

Abnormal results

Abnormal direct Coombs' test

If the DAT is positive, then there is an antibody attached to the RBCs. These are incomplete antibodies that fail to agglutinate red cell suspend in saline. But usually these antibodies are agglutinate by anti globulin.

In general, the stronger the DAT reaction (the more positive the test), the greater the amount of antibody bound to the RBCs. The DAT detects the presence of the antibody but it does not tell the doctor the source or exact type of antibody. It could be due to a transfusion reaction, autoimmune reaction, an infection, a medication, a baby-mom Rh incompatibility.

> **Note:** *If a DAT is positive due to a transfusion reaction, an infection, or drug, it will remain positive for 48 hours to 3 months. If it is positive due to an autoimmune condition, it will often be chronically positive.*

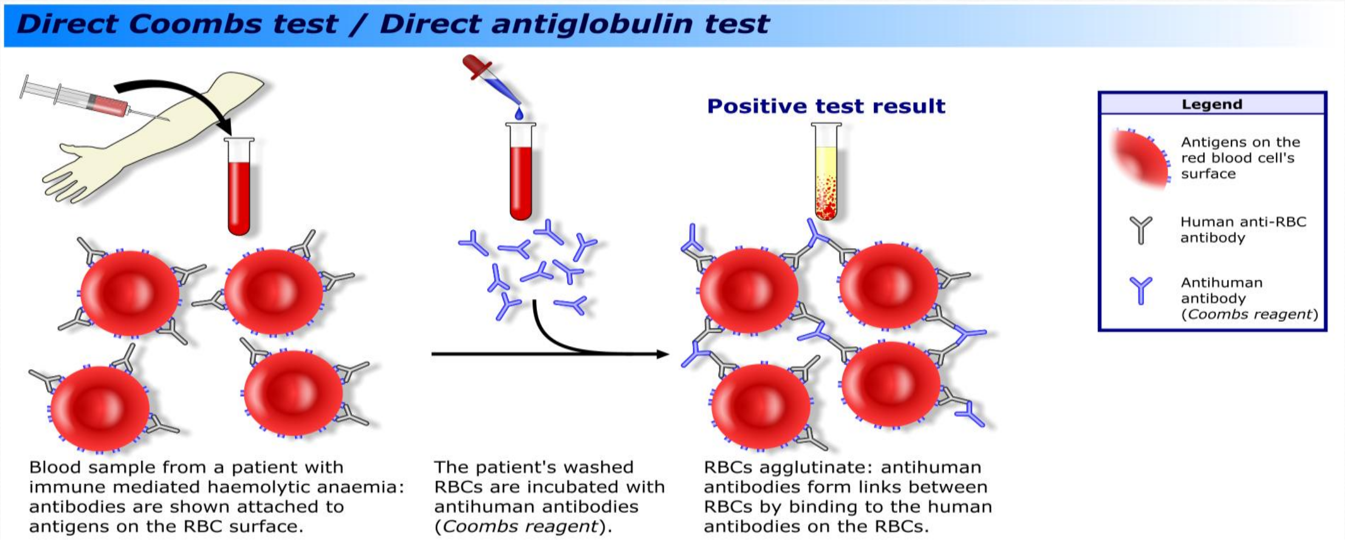
Abnormal indirect Coombs' test

If an Rh-negative mom has a negative IAT, then it is safe for a short window of time (72 hours) to give an RhIg injection to prevent antibody production. If she has a positive IAT, then the antibody or antibodies present must be identified. If there is an Rh antibody present, then the RhIg injection is not useful. If she has a different antibody, then the RhIg injection can still be given to prevent the Rh antibody.

Procedure

Direct test

- Take an EDTA sample from the patient
- Wash patient RBCs with saline (final concentration of RBCs in saline is 5%)
- Take 100 µl of patient RBCs in a test tube then add 100 µl of anti globulin reagent (Coombs reagent)
- Incubate at 37 degree for 20 minutes.
- Examine the reaction for agglutination under microscope.

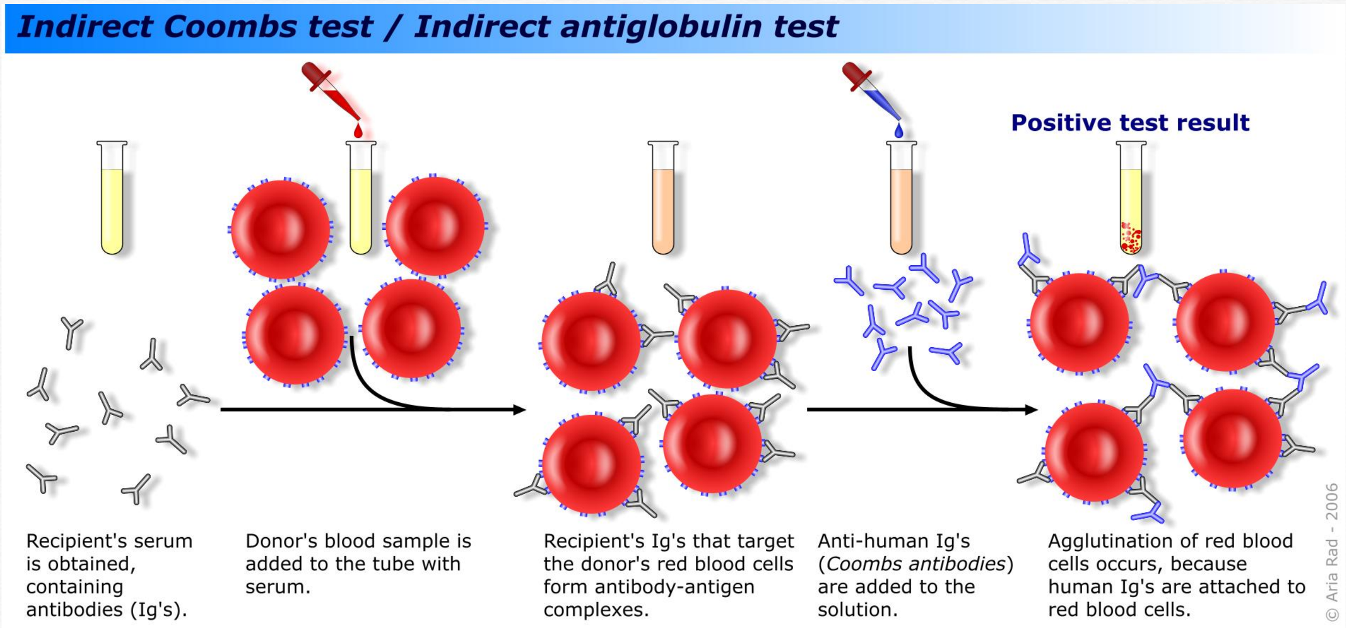


❖ ***Indirect test***

- Use RBCs of O +ve type in this test, because it do not contain antigens other than Rh on its surface.
- Wash O +ve RBCs with saline.
- Incubate O +ve RBCs with patient serum for 2 hours in incubator.

Tubes	1	2	3	4	5	6	7	Control
Serum	200 µl	100 →	200 →	200 →	200	200	200	——
Saline	——	300	200	200	200	200	200	200
O +ve	200	200	200	200	200	200	200	200
Titre	1/4	1/8	1/16	1/32	1/64	1/128	1/256	

- Take 100 µl of each tube on clean slide then add 100 µl of anti globulin reagent (Coombs reagent)
- Examine the reaction for agglutination under microscope.



Inflammatory markers

Inflammation

What is inflammation?

Inflammation is an immune system response by which the body's white blood cells and chemicals migrate out of blood vessels into the infected area to protect us from infection and foreign substances such as bacteria and viruses.

Clinical signs of inflammation

Acute inflammation is a short-term process, usually appearing within a few minutes or hours and ceasing upon the removal of the injurious stimulus. It is characterized by five cardinal signs:

redness, increased heat , swelling, pain, and loss of function.

Note *Redness and heat are due to increased blood flow at body core temperature to the inflamed site; swelling is caused by accumulation of fluid; pain is due to release of chemicals that stimulate nerve endings. Loss of function has multiple causes.*

Note *These five signs appear when acute inflammation occurs on the body's surface, whereas acute inflammation of internal organs may not result in the full set.*

Note *Inflammation can also bring on general flu-like symptoms such as fever, Loss of appetite , fatigue, loss of energy , muscle stiffness and headaches.*

Types of Inflammation

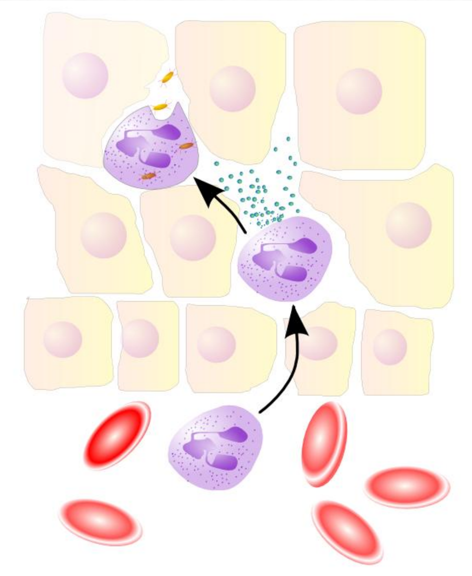
There are two basic types of inflammation – acute and chronic.

Acute inflammation

Acute inflammation is of short duration, which could be from a few minutes to a few days.

Such inflammation is caused by:

- foreign substances entering the body as bacteria
- Physical injury as burns
- A viral infection may also precipitate acute inflammation.
- Immune reactions due to hypersensitivity



Events in Acute Inflammation

A cascade of biochemical events propagates and matures the inflammatory response, involving:

- Increased blood flow due to dilation of blood vessels (arterioles) supplying the region
- Increased permeability of the capillaries, allowing fluid and blood proteins to move into the interstitial spaces
- Migration of neutrophils (and perhaps a few macrophages) out of the venules and into interstitial spaces

Chronic inflammation

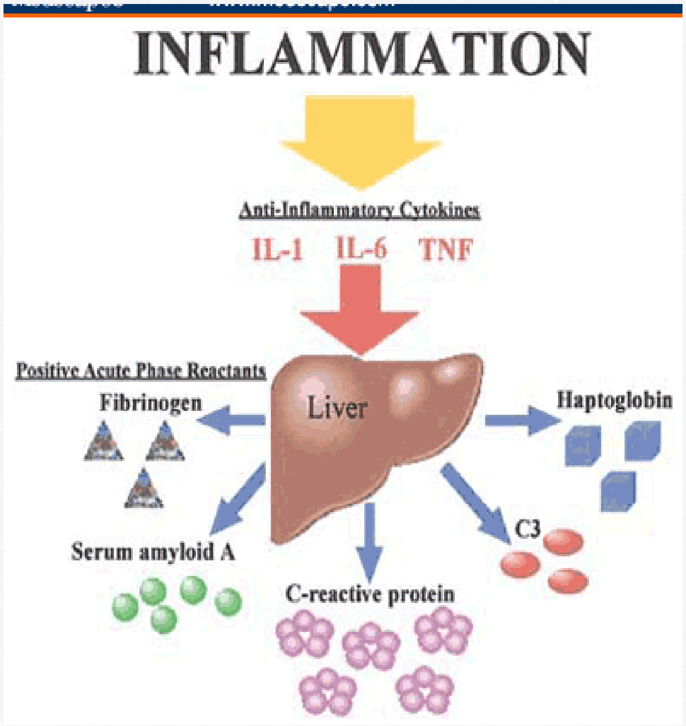
Chronic inflammation, on the other hand, is long lasting. It may persist for weeks, months or even years. Chronic inflammation may follow on from acute inflammation or exist by itself:

- An acute inflammation will become chronic if the immune system is unable to rid the body of the offending foreign agent or if the agent is constantly able to re-enter the body.
- Chronic inflammation may be the result of an auto immune disease such as rheumatoid arthritis.

Features of chronic inflammation

- The symptoms are not as severe as with acute inflammation, but the condition is persistent.
- Chronic inflammation is characterized by the dominating presence of macrophages in the injured tissue. These cells are powerful defensive agents of the body, but the toxins they release (including reactive oxygen species) are injurious to the organism's own tissues as well as invading agents. Consequently, chronic inflammation is almost always accompanied by tissue destruction.
- A key feature of chronic inflammation is collagen production. If too much collagen is formed, this can lead to the condition known as fibrosis.

Acute phase response



In response to inflammation, local inflammatory cells (neutrophil granulocytes and macrophages) secrete a number of cytokines into the bloodstream, most notable of which are the interleukins IL-1, IL-6 and IL-8, and TNF-α.

The liver responds by producing a large number of acute-phase proteins. At the same time, the production of a number of other proteins is reduced.

Acute phase proteins

Acute-phase proteins are a class of proteins whose plasma concentrations increase (positive acute-phase proteins) or decrease (negative acute-phase proteins) in response to inflammation.

Acute phase proteins help the body respond to tissue injury. For instance, some proteins may help cells destroy harmful substances, such as bacteria, while others may help the blood clot and prevent blood loss.

- **Positive acute-phase proteins**

  Positive acute-phase proteins serve different physiological functions for the immune system. Some act to destroy or inhibit growth of microbes, e.g., C-reactive protein, complement factors, ferritin (Binding iron so inhibiting microbe iron uptake), and haptoglobin (Binds hemoglobin so inhibiting microbe iron uptake).

  Fibrinogen, prothrombin: This pro-coagulant effect may limit infection by trapping pathogens in local blood clots.

- **Negative acute-phase proteins**

  Negative acute-phase proteins decrease in inflammation. Examples include albumin, transferrin, and antithrombin. The physiological role of decreased synthesis of such proteins is generally to provide amino acids in order to produce "positive" acute-phase proteins more efficiently.

Clinical significance

- Abnormal levels of acute phase proteins in the blood may indicate tissue injury or infection. and it is a useful marker of inflammation.
- Acute phase proteins can be measured indirectly as in the case of ESR test or measured directly as in the case of CRP.

Erythrocyte sedimentation rate (ESR)

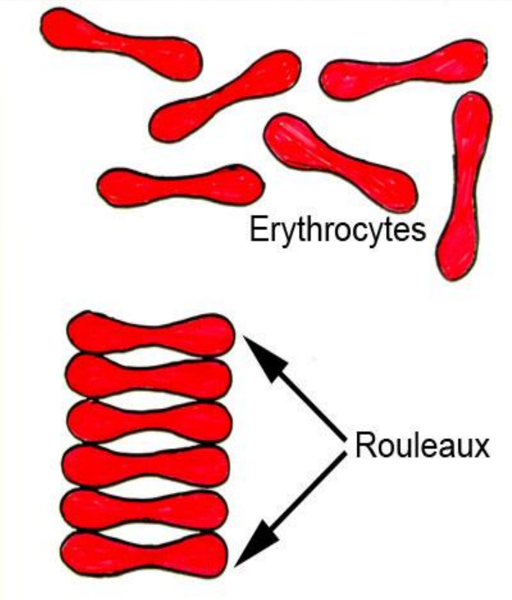
Definition

The ***erythrocyte sedimentation rate*** (ESR), also called Biernacki Reaction or Westergren test is the test that measures the distance that erythrocytes have fallen after one hour in a vertical column of anticoagulated blood under the influence of gravity.

> **Note:** *ESR is an indirect measure of the degree of inflammation present in the body.*

> **Note:** *Although there are many acute-phase proteins but ESR and CRP are the markers of choice in monitoring the acute phase is that they increase in concentration relatively high compared to basal concentration, and have a relatively short lag time from the moment of stimulus.*

Principle



The ESR is governed by the balance between:

Pro-sedimentation factors: mainly the acute phase proteins (that carry positive charge) which increase when an inflammatory process is present or with tissue damage, and

Zeta potential: the forces resisting sedimentation are the negative charge on the red cell surface causing red cells to repel each other.

- The high proportion of acute phase proteins especially fibrinogen interacts with sialic acid on the surface of RBC.
- This binding to red cell membranes thereby reduces the zeta potential thus allowing increase rouleaux formation and hence the rate of sedimentation.
- *Rouleaux formation* means the stacking up of red blood cells because of the discoid shape of the cells where the flat surface of the discoid RBCs give them a large surface area to make contact and stick to each other; thus, forming a rouleux.

Sedimentation Phases:

The phenomenon of red cell sedimentation is still only partly understood however three definite phases have been identified in the process:

The lag phase

- During the Lag Phase, the red cells form a characteristic rouleaux pattern and sedimentation is generally slow.
- This phase lasts about 10 minutes

> **Note:** *The size of the rouleaux aggregates formed in the Lag Phase is the critical factor affecting the final result of the ESR.*

The decantation Phase

- The rate accelerates in the second period and most of the settling occurs at this stage
- This phase lasts about 40 minutes

The packing phase

- The rate slows again in the final packing phase as red cell aggregates pile up at the base of the tube.
- This phase lasts about 10 minutes

Note: *The sedimentation 'rate' of the red cells is not linear. Further, the time taken for each of the three phases will differ between patients. Therefore no attempt should be made to 'estimate' the result at 1 hour by doubling the value at 30 minutes or multiplying by three after 20 minutes.*

Why It Is Done

- **To detect and monitor the activity of acute and chronic inflammations**
  ESR is called an acute-phase reactant test, meaning that it reacts to acute conditions in the body, such as infection or inflammation.

- **Diagnosis of certain conditions**
  The ESR remains an important diagnostic criterion for only two diseases polymyalgia rheumatica and temporal arteritis

  Note: *A high ESR and CRP is one of the main test results used to support the diagnosis (although cases with normal ESR and elevated CRP are reported).*

- **Monitor the response to treatment of inflammation**
  ESR has remained an appropriate measure of response to therapy for only a few diseases: temporal arteritis, polymyalgia rheumatica, and rheumatoid arthritis.
  A rising ESR can mean an increase in inflammation or a poor response to a therapy; a decreasing ESR can mean a good response.

- **Monitor the cancer statues after chemotherapy**
  In the case of Hodgkin’s disease, for example, a sustained elevation in ESR is an excellent predictor of early relapse, especially if the value fails to drop to a normal level within six months after therapy.

Sample

Citrated plasma (400 ul sodium citrate + 1.6 ml blood)

Note: *Fasting for 6 hours before ESR test is preferred*

Note: *Menstruation can interfere with the test and the accuracy of the results*

Note: *we can use EDTA solution to perform the ESR test using the same one in five dilutions (i.e. four parts blood added to one part diluent) that was used with the original technique.*

Note: *The ESR should be measured within 4 hours of collecting the blood.Even when citrated blood is stored at 4–8 C, there is a decrease in the ESR due to changes in erythrocyte shape affecting rouleaux.*

Procedure

Westergren method

The Westergren method employs a 200 mm, 2.5 mm diameter tube vertically aligned column.

Procedure

- Thoroughly mix blood specimen
  Fill the tube with anticoagulated blood to mark 0
- Insert Westergren tube gently into the rack.
- Record patient's name and position no in stand
  Set timer for 1 hour
- At the end of 1 hour read how far RBC's have settled and report in mm/hr

Normal

Normally, red cells fall slowly, leaving little clear plasma.
Results are reported as how many millimeters of this clear plasma are present at the top of the column after one hour.

Reference Ranges for the ESR in Healthy individuals	
Newborn	0 to 2 **(mm/hr)**
Neonatal to puberty	3 to 13 **(mm/hr)**
Adults age younger than 50 y Men Women	 0 to 15 **(mm/hr)** 0 to 20 **(mm/hr)**
Adults age older than 50 y Men Women	 0 to 20 **(mm/hr)** 0 to 30 **(mm/hr)**

Note:

- *ESR values tend to rise with age and to be generally higher in women.*
- *ESR values are increased in black populations.*
- *For unknown reasons, obese people have also been noted to have slightly elevated ESR.*

Interpretation

The sedimentation rate cannot be used by itself to diagnose any specific disease because:

- The ESR test is non specific marker of inflammation
- Some diseases that cause inflammation do not increase the sedimentation rate, so a normal sedimentation rate does not always rule out a disease.
- ESR is affected by many other factors such as: Gender, Age, Temperature, Smoking and Pregnancy (ESR gradually increases after the third month due elevation of fibrinogen and returns to normal after three to four weeks after delivery)

Note: *The results must be used along with other clinical findings, patient's health history, and results from other laboratory tests such as (ANA, RF, ……). If the ESR and clinical findings match, the doctor may be able to confirm or rule out a suspected diagnosis.*

Interpretation of elevated ESR

Any condition that elevates acute phase proteins may also elevate the ESR.

Moderately elevated ESR ***may indicate***:

- ***Inflammations:*** due to increased acute phase proteins and in this case the ESR increase follows a rise in body temperature and leucocytosis, peaks after several days, and usually lasts longer than the elevated temperature or white blood cells count.

  Note: *In most cases, the ESR will decrease over time once the underlying inflammation is addressed but if the patient has a chronic inflammatory disease, the ESR may fluctuate with the degree of activity your condition.*
- ***Anemia:*** due to altering the ratio of RBC to plasma (reduced hematocrit) causing increases rouleaux formation and cause red blood cell aggregates fall faster.

  Note: *Macrocytic red cells with a smaller surface-to-volume ratio also settle more rapidly.*
- Infectious diseases: e.g., typhoid fever, malaria, mononucleosis
- Peptic ulcer disease (as opposed to active inflammatory bowel disease)

- Increased plasma cholesterol level causing increased ESR due to that hypercholesterolemia increase the rouleaux formation

  Note: *moderately elevated ESR in asymptomatic individuals in the absence of other findings should not trigger an extensive laboratory or radiographic evaluation but should prompt repeat testing after several months*

Highly elevated ESR ***may indicate***:

- Autoimmune disease such as systemic lupus erythematosus
- Certain inflammatory diseases such as:

  Polymyalgia rheumatica:

  Polymyalgia rheumatica is characterized by severe aching and stiffness in the neck, shoulder girdle or pelvic girdle areas.

  Rheumatoid arthritis:

  Rheumatoid arthritis is a chronic, systemic inflammatory disorder that may affect many tissues and organs.

  Note: *The ESR traditionally has been a diagnostic parameter for rheumatoid arthritis, but it is used as a means of staging the disease rather than as one of the major diagnostic criteria.*

  Note: *In rheumatoid arthritis, there are cases where both the ESR and CRP are normal, particularly during the earliest stages of the disease. In certain patients, the reason for this appears to be an insensitivity or low level of a triggering mechanism that normally stimulates the liver to produce the inflammatory proteins necessary for ESR and CRP eleviation.*

- Graves' disease : Inflammation of the thyroid gland
- Chronic infective conditions like tuberculosis
- Chronic kidney failure: in chronic renal disease, changes in the ESR appear to be due to alterations in fibrinogen concentrations and the constituents of uremic plasma, independent of the degree of anemia.

  Note: *highly elevated ESR in asymptomatic patient may be followed by some tests such as Hematology profile, creatinine & urea, urinalysis, serum and urine protein electrophoresis and occult blood testing of stool*

Extremely high ESR ***(greater than 100 mm/hr) may indicate***:

- Some severe infections (such as osteomyelitis or endocarditis)
- Certain inflammatory diseases such as

  Temporal arteritis

  - It is usually characterized by headaches, visual disturbances, a tender, reddened or nodular temporal artery, facial pain and jaw claudication.
  - The average ESR was greater than 90 mm per hour in patients who had temporal arteritis, with values exceeding 30 mm per hour in 99 percent of the cases however, an occasional patient may present with a normal value.
- Certain types of cancer (especially lymphoma, Waldenstrom’s macroglobulinemia, multiple myeloma or chronic lymphocytic leukemia)

  Note: *ESR May be elevated in multiple myeloma when CRP normal*

  Note: *Because a notable number of patients with an ESR greater than 100 mm per hour have myeloma serum protein electrophoretic studies should be included to diagnose the condition.*

Note: *In patients with solid tumors, an ESR greater than 100 mm per hour usually indicates metastatic disease.*

Note: *In oncology, a high ESR has been found to correlate with overall poor prognosis for various types of cancer, including Hodgkin's disease, gastric carcinoma, renal cell carcinoma, chronic lymphocytic leukemia, breast cancer, colorectal cancer and prostate cancer.*

Interpretation of decreased ESR

Although a low ESR is not usually important, it can be associated with a number of blood diseases in which red blood cells have an irregular or smaller shape that causes slower settling such as:

- In patients with polycythemia, too many red blood cells decrease the compactness of the rouleaux network and artifactually lower the ESR.
- Spherocyosis and Sickle cell anemia (unless there is severe anemia.)
- Low value may be found during severe liver disease due to decreased protein synthesis
- Hypofibrinogenemia and Hypogammaglobulinemia may cause a marked decrease in the ESR.
- A low value may also occur with an increased blood sugar level due to increased blood viscosity.

What Affects the Test

Factors cause falsely lower ESR

- Correct mixing of the blood sample at vein puncture to prevent clotting is essential (inadequate anticoagulation of blood sample will consume fibrinogen and falsely lower the ESR)
- Excessive anticoagulant cause false low results
- The test should be set up within two hours after the blood sample is collected to avoid a false low sedimentation rate.
- Lower room temperature cause false low results.
- Vibration can seriously affect the ESR (reduce the ESR) so ensure that the bench top is not in contact with machinery (especially centrifuges).
- Dirty tubes can decrease the rate and cause false low results.
- Air bubbles can interfere with the test result and cause false low results

Factors cause falsely higher ESR

- The ESR measuring tube must be kept perfectly vertical (slight deviations from the absolute vertical can accelerate the rate by 30 percent and increase the result).
- The ESR must be performed at room temperature (18 - 22°C) (higher temperature cause false high results due to reduction in plasma viscosity)
- Keep the stand away from windows, or direct sunlight (due to high temperature).

Note:

- *Mixing of the blood sample immediately before setting up the test is one of the most important factors influencing the ESR result.*
- *Ensure you read the result of each test at exactly 1 hour from when you set up the tube in the rack*
- *Do not pick up the stand to read results as this will affect other tests in progress. Bring the eye to the level of the top of the red cells to read.*

Disadvantage

- The ESR is a nonspecific marker of inflammation because increases do not tell the doctor exactly where the inflammation is in your body or what is causing it
- The use of the ESR as a screening test in asymptomatic persons is limited by its low sensitivity and specificity.
- The usefulness of this test has decreased as new methods of evaluating disease have been developed.
- The ESR is affected by a variety of factors, including size/shape of red blood cells, plasma composition, and fluid status.

C - reactive protein

Definition

C-reactive protein (CRP) is a protein synthesized by the liver in response to to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages.during inflammation (i.e. C-reactive protein is an acute-phase protein).
Its physiological role is to bind to phosphocholine expressed on the surface of dead cells (and some types of bacteria) in order to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages (***opsonin mediated phagocytosis***), which express a receptor for CRP.

> **Note:** *CRP rises up to 50,000-fold in acute inflammation, such as infection. It rises above normal limits within 6 hours, and peaks at 48 hours.*

Why It Is Done

A C-reactive protein (CRP) test may be done to:

- Help monitor the activity of certain inflammatory conditions, including inflammatory bowel disease, temporal arteritis, rheumatoid arthritis. CRP levels decrease in these conditions after treatment.
- Check for infection after surgery. CRP levels normally rise within 2 to 6 hours of surgery and then go down by the third day after surgery. If CRP levels stay elevated 3 days after surgery, an infection may be present.
- Check to see how well treatment is working, such as treatment for infection. CRP levels go up quickly and then become normal quickly if you are responding to treatment measures.
- The C-reactive protein test can also be used to check your risk of developing coronary artery disease, a condition in which the arteries of your heart are narrowed. Coronary artery disease can eventually lead to a heart attack.

> **Note:** *It must be noted that even in known cases of inflammatory disease, such as rheumatoid arthritis and lupus, a low CRP level is possible, and is not indicative of no inflammation.*

Procedure

Latex agglutination test

Qualitative assay

Assay: mix equal volumes of both reagent and serum (for example 50 ul + 50 ul) on the black slide and rotate carefully for two minutes.

Results:

- **Positive**: The reagent will agglutinate (clump) in the presence of CRP.
- **Negative**: No agglutination generally indicates absence of CRP

Semi-quantitative assay

- Make a serial dilution as follow:

Serum	50 ul →			
saline	50 ul	50 ul	50 ul	50 ul
dilution	1/2	1/4	1/8	1/16
Titre	6	12	24	48

- Using the first tube mix equal volumes of both reagent and serum (for example 50 ul + 50 ul) on the black slide and rotate carefully for two minutes , if positive result appear then make the same test using the second tube.
- The titer is expressed as the highest dilution showing agglutination (positive result)

Normal

Normal concentration in healthy human serum is usually lower than 6 mg/L, slightly increasing with ageing.

Interpretation

Elevated CRP levels can be caused by any condition that results in acute inflammation, including:

- Bacterial infections
- Infection of a bone (osteomyelitis).
- Inflammatory bowel disease
- Rheumatic fever
- Rheumatoid arthritis
- Temporal arteritis
- Heart attack
- Cancer of the lymph nodes (lymphoma).

What Affects the Test

- A positive CRP can be detected during the last half of pregnancy or with the use of oral contraception.
- Vigorous exercise can cause mildly elevated CRP levels.
- Women who are on hormone replacement therapy (HRT) (i.e., estrogen) may have increased CRP levels.
- Women have an intrauterine device (IUD) may have increased levels.
- Drugs such as nonsteroidal anti-inflammatory medications, salicylates, and steroids can cause levels to decrease.

Disadvantage

The measurement of CRP does not reflect where the inflammation is.

Comparison between ESR & CRP

Recent studies tend to favor CRP over ESR, mainly because:

- CRP levels in the blood rise more quickly after the inflammatory or infective process begins (ESR does not change as rapidly as does CRP).
- CRP appears and disappears more quickly than changes in ESR. Therefore, CRP level may drop to normal following successful treatment, whereas ESR may remain elevated for a longer period.
- CRP is not affected by as many other factors as is ESR, making it a better marker of inflammation.

  Note: *It is therefore recommended that CRP and ESR are tested simultaneously, which will result in a higher sensitivity and specificity for diagnosis.*

Rheumatology profile

Joint structure

A joint is the location at which two or more bones make contact. They are constructed to allow movement and provide mechanical support.

Joints are mainly classified structurally and functionally. Structural classification is determined by how the bones connect to each other, while functional classification is determined by the degree of movement between the articulating bones. In practice, there is significant overlap between the two types of classifications.

Structural classification

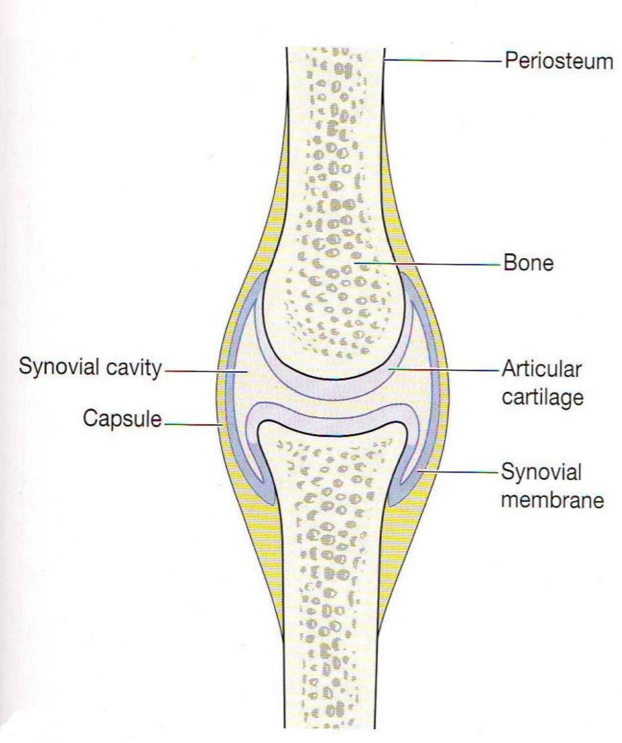
Structural classification names and divides joints according to how the bones are connected to each other. *There are three structural classifications of joints:*

- **Fibrous joint** - joined by dense irregular connective tissue that is rich in collagen fibers
- **Cartilaginous joint** - joined by cartilage
- **Synovial joint** - not directly joined - the bones have a synovial cavity and are united by the dense irregular connective tissue that forms the articular capsule that is normally associated with accessory ligaments.

Functional classification

Joints can also be classified functionally, by the degree of mobility they allow:S

- **Synarthrosis** - permits little or no mobility. Most synarthrosis joints are fibrous joints (e.g., skull sutures).
- **Amphiarthrosis** - permits slight mobility. Most amphiarthrosis joints are cartilaginous joints (e.g., vertebrae).
- **Diarthrosis** - permits a variety of movements. All diarthrosis joints are synovial joints (e.g., shoulder, hip, elbow, knee, etc.), and the terms "diarthrosis" and "synovial joint" are considered equivalent.



Synovial joint

A Synovial joint, is the most common and most movable type of joint in the body of a mammal. As with most other joints, synovial joints achieve movement at the point of contact of the articulating bones.

Synovial joints contain the following structures:

- **Synovial cavity:** all diarthroses have this characteristic space between the bones that is filled with synovial fluid.
- **Articular capsule:** the fibrous capsule, continuous with the periosteum of articulating bones, surrounds the diarthrosis and unites the articulating bones; the articular capsule consists of two layers:

  (1) the outer fibrous membrane that may contain ligaments and

  (2) the inner synovial membrane that secretes the lubricating, shock absorbing, and joint-nourishing synovial fluid.

- **Articular cartilage:** the bones of a synovial joint are covered by this layer of hyaline cartilage that lines the epiphyses of joint end of bone with a smooth, slippery surface that does not bind them together; articular cartilage functions to absorb shock and reduce friction during movement.

Arthritis

Arthritis is a form of joint disorder that involves inflammation of one or more joints. Regardless of the type of arthritis, the common symptoms for all arthritis disorders include varied levels of pain, swelling, joint stiffness and sometimes a constant ache around the joint(s).
There are over 100 different forms of arthritis. The most common form are:

Osteoarthritis (خشونه الركبه)

Osteoarthritis is the most common form of arthritis. It can affect both the larger and the smaller joints of the body, including the hands, feet, back, hip or knee. The disease is essentially one acquired from daily wear and tear of the joint, however, osteoarthritis can also occur as a result of injury. Osteoarthritis begins in the cartilage and eventually leads to the two opposing bones eroding into each other. Initially, the condition starts with minor pain while walking but soon the pain can be continuous and even occur at night. The pain can be debilitating and prevent one from doing some activities. Osteoarthritis typically affects the weight bearing joints such as the back, spine, and pelvis. Unlike rheumatoid arthritis, osteoarthritis is most commonly a disease of the elderly.

More than 30 percent of females have some degree of osteoarthritis by age 65.

Risk factors for osteoarthritis include: prior joint trauma, obesity, sedentary lifestyle.

Osteoarthritis, like rheumatoid arthritis, cannot be cured but one can prevent the condition from worsening. Weight loss is the key to improving symptoms and preventing progression. Physical therapy to strengthen muscles and joints is very helpful. Pain medications are widely required by individuals with osteoarthritis.

Gouty arthritis

Gout is caused by deposition of uric acid crystals in the joint, causing inflammation. In the early stages, the gouty arthritis usually occur in one joint, but with time, it can occur in many joints and be quite crippling. The joints in gout can often become swollen and lose function.

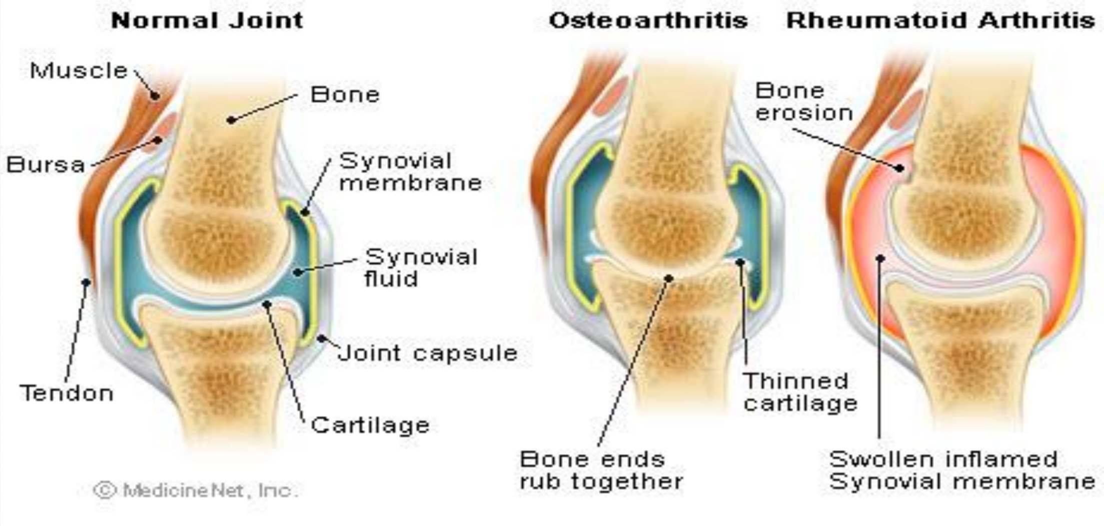
Rheumatoid arthritis

Rheumatoid arthritis (RA) is an chronic autoimmune disease that causes chronic inflammation of the joints. Rheumatoid arthritis can also cause inflammation of the tissue around the joints, as well as in other organs in the body. Autoimmune diseases are illnesses that occur when the body's tissues are mistakenly attacked by their own immune system.

What is the symptoms of rheumatoid arthritis?

The joint inflammation of rheumatoid arthritis causes swelling, pain, stiffness, and redness in the joints. The inflammation of rheumatoid disease can also occur in tissues around the joints, such as the tendons, ligaments, and muscles.
In some patients with rheumatoid arthritis, chronic inflammation leads to the destruction of the cartilage, bone, and ligaments, causing deformity of the joints. Damage to the joints can occur early in the disease and be progressive.

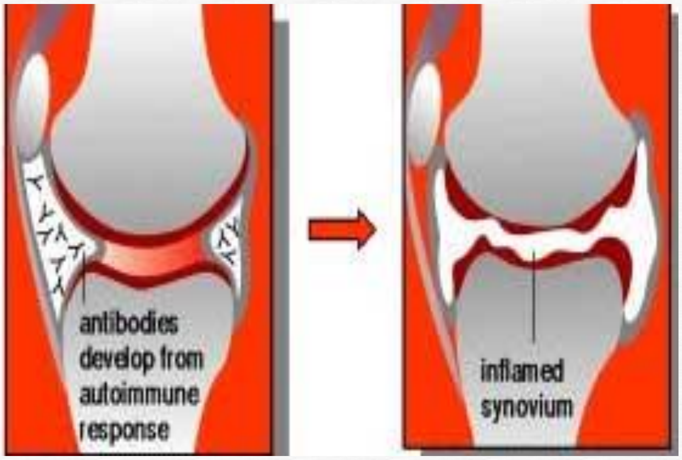
Note: *The stiffness seen in active RA is typically worst in the morning and may last one to two hours or throughout the entire day. This long period of morning stiffness is an important diagnostic clue*



► What causes rheumatoid arthritis?

RA is classified as an autoimmune disease, which develops because certain cells of the immune system don't work properly and begin attacking healthy joints.

Regardless of the exact trigger, the result is an immune system that is geared up to promote inflammation in the joints and occasionally other tissues of the body. Immune cells, called lymphocytes, are activated and chemical messengers (cytokines, such as tumor necrosis factor/TNF, interleukin-1/IL-1, and interleukin-6/IL-6) are expressed in the inflamed areas.



► How is rheumatoid arthritis treated?

There is no known cure for rheumatoid arthritis, but many different types of treatment can alleviate symptoms and/or modify the disease process.

The goal of treatment is two-fold:

- Alleviating the current symptoms, and
- Preventing the future destruction of the joints

Two classes of medications are used in treating rheumatoid arthritis:

- **Fast-acting "first-line drugs"**

The first-line drugs, such as aspirin and cortisone (corticosteroids), are used to reduce pain and inflammation.

- **Slow-acting "second-line drugs"**

(also referred to as disease-modifying antirheumatic drugs or DMARDs). The slow-acting second-line drugs, such as methotrexate, and hydroxychloroquine (Plaquenil), promote disease remission and prevent progressive joint destruction, but they are not antiinflammatory agents.

Methotrexate is an immune-suppression drug. It can affect the bone marrow and the liver. All patients taking methotrexate require regular blood tests to monitor blood counts and liver function tests.

Rheumatoid Factor

overview

RF is an autoantibody, an IgM (immunoglobulin M) protein that is produced by the body's immune system. Autoantibodies usually attack the patient's own tissues, mistakenly identifying them as "foreign." While the role of RF is not well understood, it is not thought to directly cause joint damage but to promote the body's inflammation reaction, which in turn contributes to autoimmune tissue destruction.

Not all people with rheumatoid arthritis have detectable rheumatoid factor. Those who do not are said to be "seronegative".

Why It Is Done

The rheumatoid factor (RF) test is primarily used to help diagnose rheumatoid arthritis (RA) and to distinguish it from other forms of arthritis and other conditions that cause similar symptoms of joint pain, inflammation, and stiffness as gout.

Sample

Only serum sample

Note:

- *The specimens may be stored at 2-8ºC for 48 hours. For longer periods of time freeze serum at or below –20ºC*
- *Do not use haemolysed, lipaemic , contaminated serum specimens or high lipimic samples which may cause a falsely high rheumatoid factor (RF) result.*

Procedure

Principle

The earliest tests, and those still most widely used clinically rely on the agglutinating properties of the IgM class of RF. IgG, usually human or rabbit, is bound to a particulate carrier, and the presence of RF is then detected by agglutination or flocculation of the respective indicator system. Carrier particles frequently used include latex, and erythrocytes.

Semi-quantitative analysis

to determine the antibody content of a serum involves serial dilutions of the serum and determination of an end point (the last dilution at which agglutination can be visualized). The reciprocal of this dilution is known as "***antibody titer***".

Latex agglutination test

Qualitative assay

Positive: The reagent will agglutinate (clump) in the presence of RF.

Negative: No agglutination generally indicates absence of RF

Semi-quantitative assay

The titer is expressed as the highest dilution showing agglutination

Serum	50 ul →			
saline	50 ul	50 ul	50 ul	50 ul
dilution	1/2	1/4	1/8	1/16
Titre	16	32	64	128

Normal

Normal levels of RF in adults are < 8 mg/l.

Normally about 5% to 10% of people over age 65 have an elevated RF level.

Interpretation

- In patients with symptoms and clinical signs of rheumatoid arthritis, the presence of significant concentrations of RF indicates that it is likely that they have RA.
- High concentrations of RF are often found in people with active cases of rheumatoid arthritis, and it correlates with the severity of signs or symptoms (Prognosis worse with high titer at onset).
- RF Incidence increases with duration of disease
  - Three months: 33%
  - Six months: 45%
  - One year: 75%
  - Eighteen months: 90%

Note: *A negative RF test does not rule out RA. About 20% of patients with RA will be persistently negative for RF and/or may have very low levels of RF.*

Note: *The RF test is sensitive but nonspecific. It is most closely associated with rheumatoid arthritis (RA) but may also be present in a variety of other conditions such as:*

- *Autoimmune disease*
  - ✓ *Sjogren's Syndrome (75 to 95%)*
  - ✓ *Systemic Lupus Erythematosus (15 to 35%)*
  - ✓ *Scleroderma (20 to 30%)*
- *Infections*
  - ✓ *Osteomyelitis*
  - ✓ *Tuberculosis*
  - ✓ *Syphilis*
  - ✓ *parasitic diseases (e.g., malaria, schistosomiasis, filariasis)*
  - ✓ *Hepatitis (Acute and Chronic) and hepatic cirrhosis (10% to 40%)*
  - ✓ *Leukemia, and multiple myeloma*

Note: *RF test is negative in osteoarthritis, rheumatic fever, and gout*

Anti-cyclic citrullinated peptide antibody

overview

Anti-CCP, which stands for ***anti-cyclic citrullinated peptide antibody***, is a new test for RA.
Anti-citrullinated protein antibodies (ACPA) or anti-cyclic citrullinated protein antibodies (anti-CCP) are autoantibodies (antibodies directed against one or more of an individual's own proteins) that are frequently detected in the blood of rheumatoid arthritis patients.

Role in pathogenesis

- During inflammation, Citrulline is formed by de-imination of arginine residues in several proteins by the action of enzyme peptidylarginine deiminase (PAD).
- PAD isoenzymes are abundant in the inflammatory RA synovium and cause the local citrullination of synovial proteins.
- If the shape of these proteins are significantly altered, the proteins may be seen as antigens by the immune system, thereby generating an immune response.

ACPAs have proved to be powerful biomarkers that allow the diagnosis of rheumatoid arthritis (RA) to be made at a very early stage.

Sample

Serum sample is used (separate serum from cells within 2 hours of collection)

Unacceptable conditions:

Urine or plasma contaminated, hemolyzed, grossly icteric, heat-inactivated, or severely lipemic specimens.

Stability:

After separation from cells: Ambient: 48 hours; Refrigerated: 2 weeks; Frozen: 1 year (avoid repeated freeze/thaw cycles).

Procedure

Antibodies to citrullinated peptides (CCP) detected by ELISA

Interpretation

Anti-cyclic citrullinated peptide (anti-CCP), IgG antibodies are present in about 69-83 percent of patients with rheumatoid arthritis (RA) and have specificities of 93-95 percent.

According to the American College of Rheumatology (ACR), CCP antibodies may be detected in about 50-60% of patients with early RA, as early as 3-6 months after the beginning of symptoms.

> **Note:** *Some patients may have a negative RF test with clinical signs of RA. The anti-CCP is used to confirm the presence of RA before it has developed adequately to show positive in other tests.*

Early detection and diagnosis of RA allows doctors to begin aggressive treatment of the condition, minimizing the associated complications and tissue damage.

Identify Rheumatoid Arthritis With Anti-CCP Blood Test Results

Positive anti-CCP and positive RF

Consider a positive anti-CCP blood test and a positive RF result a likely indication that rheumatoid arthritis is the appropriate diagnosis.

A positive for both tests are an indication that a more severe form of RA may develop.

Positive anti-CCP and negative RF

Take note that in a patient with positive anti-CCP, a negative RF and clinical signs of RA, the diagnosis will likely be RA. If the disease is not actively present at the time, it is probable that it will develop in the future.

Negative anti-CCP and positive RF

If the anti-CCP is negative and the RF is positive the doctor must rely more on symptoms and signs of rheumatoid arthritis for diagnosis.

Negative anti-CCP and negative RF

Find it less likely that rheumatoid arthritis is present when both the anti-CCP and RF are read as negative.

Advantage of anti-CCP test

- Anti CCP-2 antibodies show a great promise as a diagnostic marker of RA as they can be detected very early in RA.
- They have also shown the ability to distinguish between erosive and non erosive disease, making them a good prognostic marker.

Several observations have indicated that anti-CCP positive early RA patients may develop a more erosive disease than those without anti-CCP.

- Use the results of the anti-CCP blood test to distinguish the type of arthritis. Anti-CCP is specific to identifying rheumatoid arthritis from conditions such as SLE.